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MANUAL OF PRACTICAL MEDICAL AND PHYSIOLOGICA CHEMISTRY

BY

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WITH ILLUSTRATIONS

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1892

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PREFACE.

DURING the summer of 1887, the author, who had been recently appointed to take charge of the new chemical laboratory at the College of Physicians and Surgeons, in company with Prof. C. F. Chandler, visited or interrogated most of the leading medical colleges both in this country and abroad, with a view to determine the course of practical chemical instruction to be adopted for the coming term. It was found that in every case where the practical instruction given was more than merely urine analysis, with sometimes a little toxicology, it consisted of a regular course of qualitative, and perhaps some quantitative, analysis. This method it was decided not to adopt. There seemed to be too much pure chemistry and too little medicine in such a course for students who were studying to be physicians, and not chemists. So, with the advice and assistance of Dr. Chandler, a course was prepared, limited, by the size of the laboratory and the length of the term, to thirty lessons, in which, as far as possible, every subject and every test had some bearing upon the student's other work.

The lessons for this course of laboratory work were published in pamphlet form in 1889, and the present book contains the same lessons revised, and amplified with a descriptive and explanatory text.

It will be noticed that these lessons not only deal with true physiological chemistry, with the foodstuffs and their products of assimilation, and with the different fluids and tissues of the body, but that, wherever possible, particular attention has been paid to the latest clinical tests. Special care has been bestowed on the tests of breast milk, for which the author wishes to thank Dr. L. Emmett Holt, and on those of the gastric juice, largely taken from the excellent paper of Dr. F. P. Kinnicutt.

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Some of the illustrations will, it is hoped, prove of interest. The drawings of crystals, in the first half of the book, were inserted mainly with the idea of accustoming the student to the free use of the microscope. It will be noticed, however, that most of these crystals also occur among the different urinary deposits described in the last part. Attention is called to the microphotographs of blood-cells, Plates IV. and V., inserted by the courtesy of Dr. John S. Billings, U.S.A., which illustrate the extreme difficulty, if not the impossibility, of distinguishing between human blood and that of some other mammalia. Special thanks are due to Dr. E. G. Love for his fine drawings of starch granules, Figs. 1 and 2; to Dr. H. Holbrook Curtis for the use of Plates VII. and VIII., taken from his translation of Hoffmann and Ultzmann's "Urine Analysis;" and to Messrs. Appleton & Co. for the loan of several cuts from Frey's "Histology" and Flint's "Physiology."

Besides gratefully acknowledging his indebtedness to Dr. Chandler, the author wishes to return his hearty thanks to Dr. John G. Curtis, Dr. T. M. Prudden, and Dr. William G. Thompson for their kind assistance in looking over and criticising the proof-sheets; and also to his many friends on the former and present House Staffs of the hospitals in this city, who have never, from the first year of the laboratory, failed to assist him in every possible way with both material and information.

College Physicians and Surgeons,
August 26th, 1892.

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PART I.

THE CARBOHYDRATES.

THE CARBOHYDRATES.

INTRODUCTION.

Occurrence.—The name Carbohydrate has been given to a very important series of Proximate Principles, found chiefly in the vegetable kingdom, where they compose by far the greater bulk of the plant tissue, but also found in smaller quantities in the animal kingdom.

Composition.—The carbohydrates contain no nitrogen—only carbon, hydrogen, and oxygen. As the name signifies, the last two are in the proportion to form water. There are, however, many substances of a similar composition, $C_mH_{2n}O_n$, such as acetic acid, $C_2H_4O_2$, lactic acid, $C_3H_6O_3$, pyrogallic acid, $C_6H_6O_3$, and others, which have nothing else in common with this group.

In order to belong to the carbohydrates, a substance must satisfy the following conditions:

1st. It must contain at least six atoms of carbon and, in general, five atoms of oxygen.

2d. It must be composed of carbon, hydrogen, and oxygen, only, with two atoms of hydrogen for every one of oxygen.

3d. Either unchanged, or after treatment with various reagents, such as heat, acids, ferments, etc., it must have at least some of the following properties:

(*a*) A sweet taste.

(*b*) The power to rotate a beam of polarized light.

(*c*) The power to reduce solutions of certain metals, such as copper, bismuth, mercury, etc.

(*d*) The property of undergoing alcoholic fermentation.

General Properties.—The carbohydrates are, almost without exception, neutral in reaction. They form some loose chemical combinations with other substances, principally bases, a fact which is sometimes used as a means of purifying them.

Many of them crystallize readily, like cane and milk sugar and dextrose; others can be crystallized, but only with great difficulty. Still others of the series, like starch and cellulose, are distinctly colloid in both appearance and properties. These latter are of a more complex structure than the former.

Their exact chemical composition is at present but little known.

Classification.—Carbohydrates can be classified according to their composition, dividing them with reference to the number of times that they contain a group of six carbon atoms.

On this basis the following table can be made:

I. MONO-SACCHARIDS, or GLUCOSES.—$C_6H_{12}O_6$.

Dextro-glucose (dextrose, grape sugar).
Lævo-glucose (lævulose).
Galactose.
Sorbose, and others, of less importance.

II. DI-SACCHARIDS, or SACCHAROSES.—$C_{12}H_{22}O_{11}$.

Sucrose (saccharose, cane sugar).
Lactose (milk sugar).
Maltose (malt sugar) and others.

III. POLY-SACCHARIDS.—$_n(C_6H_{10}O_5) \pm {}_m(H_2O)$.

1st. **Crystallizing.**

Raffinose, *lactosin*, and others, all of little importance.

2d. **Colloid.**

Cellulose.
Starch, *dextrins*, and other derivatives, all polarizing to the right.
Inulin, and its derivatives, polarizing to the left.
Glycogen.
Arabinose, *gums*, etc.

In general it may be said that the last group, of poly-saccharids, is by far the most complex in character. Its members may be readily broken down into simpler carbohydrates by a variety of reagents, while, so far, with but few exceptions, we have been unable to build them up without the agency of plant life.

The second group, of di-saccharids, although simpler than the last, and in some cases made from them, are still far more

complex than the glucoses, into which they can be readily broken down; while any attempt to break down the latter into still simpler compounds only results in destroying their composition, and decomposing them into substances no longer to be considered carbohydrates.

LESSON I.

CELLULOSE AND STARCH.

CELLULOSE.—$(C_6H_{10}O_5)_n$.

Occurrence.—Cellulose is present in all the tissues of the higher plants, and in most of the lower forms of vegetable life. It may be considered as the framework, the skeleton, of these plants. In the more delicate tissues it is found perfectly pure, forming the walls of the minute cells, which act as the basis for all parts of the structure. This is well seen under the microscope in studying the potato section, where the round and oval starch grains lie inside the delicate threads of cellulose which define the cells. In the solid portions of the plant which we call wood the cellulose, still forming the framework, is firmer in structure, and more or less contaminated with thickening and stiffening materials, both organic and inorganic. It is worth noticing, however, that even in those parts of the plant like the trunks of trees, which have to stand the greatest stress, the percentage of mineral matter rarely runs over 6 or 7%, while in the bones of animals about two-thirds of their weight is mineral matter. The cellulose is probably formed in the plants from the various carbohydrates, such as cane sugar, dextrin, and glucose, dissolved in the sap.

Preparation.—Cellulose is prepared in a pure state from almost any vegetable tissue by simply reducing it to a pulp and then washing away the various impurities, starch, gums, fats, and salts, which may be present. In our experiment the grating process breaks up the cells and sets the starch granules free. Then, by washing and straining, the threads of cellulose which formed the cell walls are finally left in a more or less pure condition.

Cellulose is thus prepared from wood, flax, cotton, hemp, and other similar materials, on a large scale, and comes into commerce in the form of paper, linen, cotton, cordage, etc. The finer vari-

eties of filter paper and of absorbent cotton are, excepting for the moisture they contain, almost chemically pure cellulose.

Composition.—Cellulose is composed of 44.5% carbon, 6% hydrogen, and 49.5% oxygen. Its exact chemical formula is not known, but it is some multiple of the formula $C_6H_{10}O_5$.

Properties.—In a pure state it consists of extremely fine, colorless, flexible, slightly elastic fibres, which mat together to form the paper and cotton, with which we are familiar. These fibres are doubly refracting, often giving a play of colors when seen by polarized light.

When perfectly pure, cellulose is quite unaltered by either air or moisture. As we find it in nature, however, it is invariably associated with more or less other organic matter, carbohydrates, proteids, etc., and then it gradually undergoes decomposition by the aid of the lower organisms. It is insoluble in water, and is affected but little by most of the ordinary reagents. Strong alkalies, like caustic potash or soda, soften it and alter its structure more or less, without, however, actually dissolving it. Strong acids also affect it to some extent, and concentrated sulphuric acid decomposes it completely, even in the cold, breaking it down into dextrin and glucose, and some dark-colored derivatives, belonging to the caramel group.

When as paper, it is exposed to the action of sulphuric acid only for a moment, a curious change takes place; part of the cellulose is changed to hydro-cellulose, a jelly-like substance, which settles between the fibres of the paper and thus forms the so-called "Parchment Paper," *v.* Lesson XI. This hydro-cellulose, or amyloid as it is sometimes called, is colored blue by iodine.

Cellulose is completely dissolved by a strong solution of cupric hydrate in ammonic hydrate, from which it is reprecipitated by neutralizing with strong acid, or even by diluting greatly with water. It is also soluble in an acid solution of zinc chloride.

Excepting, possibly, in some of the most delicate tissues, *e.g.*, in young vegetables, cellulose is but little affected by the human digestive ferments. Herbivorous animals, however, are able to digest it in certain forms without much difficulty.

Uses.—It is used enormously in the arts, either in a comparatively pure condition, as cotton, linen, rope, and paper, or

mixed with other materials, as papier maché and similar products, or in the form of wood. It also serves as a basis for the manufacture of certain nitro-compounds, such as nitro-cellulose or gun-cotton, and of their derivatives, celluloid, lignoid, and the like.

STARCH.—$(C_6H_{10}O_5)_n$.

Occurrence.—Starch is found in small quantities in the leaves, bark, stem, and in fact almost every organ of the higher plants. It occurs in much greater abundance in the seeds, roots, and tubers, where it is stored up as food for the young plant. In a natural condition potatoes contain about 20% of dry starch and nearly 70% of water; in wheat, corn, and other cereals the percentage of starch varies from 50%-70%, according to their quality and dryness.

It is generally considered that starch is formed, directly or indirectly, in the leaves of all green plants by sunlight, acting through chlorophyll on water and the carbon dioxide of the atmosphere. Oxygen is liberated at the same time, perhaps according to the imperfect formula

$$6CO_2+5H_2O=C_6H_{10}O_5+6O_2.$$

Preparation.—Starch is prepared from either potatoes or grain, by a process similar in every respect to that in the lesson. It always occurs in the form of minute microscopical particles, filling more or less completely the cell wall of cellulose. To extract the starch it is simply necessary to break up these cells by crushing or grating, after which the starch granules can be washed out, strained from the coarser fibres of cellulose and from other débris, and finally washed by decantation or otherwise. To obtain a very pure product this washing is conducted with great care, and with the addition of a little soda to dissolve off the last traces of proteid and fatty material.

Composition.—Commercial starch always contains some water, as well as faint traces of salts, albumin, fat, etc. When pure its composition is the same, so far as we can tell, as that of cellulose, and we give it the same indefinite formula of $(C_6H_{10}O_5)_n$.

Properties.—The granules of every variety of starch, while differing from each other in size (0.003 to 0.1 millim.), in shape,

and in appearance, still possess the same general structure. They seem to be composed of layers, overlapping one another, and covering in a central portion. The generally received theory is that two entirely different substances enter into the composition of these granules. The one, insoluble starch or starch cellulose, comprises the outer layer of thin husks, impermeable to cold water, and shielding the far larger amount (over 95% of the whole), of the more valuable soluble starch or starch granulose within. Hence it is that the starch grains are quite insoluble in cold water, but when treated with hot water or with alkalies the layers of starch cellulose are loosened and finally peel off, scattering through and thickening the liquid, and exposing the soluble granulose within.

Hot dilute acids and also the more powerful ferments, like diastase and amylopsin, are able to penetrate the outer covering and to disintegrate and change to maltose the raw starch. This occurs in nature, when the seeds that have lain in the ground all winter begin to germinate in the spring, and the diastase ferment, then formed, digests the stored-up starch, and thus nourishes the young plant till it can take care of itself. The action, however, even of the diastase, is far more rapid if the starch grains have been first disintegrated by boiling.

An important property of starch is the facility with which various agents—heat, acids, ferments, etc.—break it down into dextrin, and into maltose, or glucose. This is a necessary part of the process of digestion, for starch as such is a "colloid" or non-dialyzing substance, and before it can be assimilated must be changed to a "crystalloid" body like the two last mentioned. In the human body this is done to a slight extent by the saliva, but principally by the amylopsin ferment of the pancreatic juice.

Tests.—The principal test for starch is the formation of a blue compound known as iodide of starch, whenever free iodine is allowed to act on starch granulose. The cellulose layers in raw starch do not change color themselves, but allow the iodine to penetrate through them.

It is probable that this substance is a pure chemical compound of iodine with the starch molecule, and that the presence of some hydriodic acid is necessary for its produc-

tion. It is purplish blue when wet, and brown when dry; it contains, when pure, about 18% of iodine. It is not a stable compound. Heat is sufficient to decompose it into starch and free iodine, and if the heat is continued long enough all the iodine is driven off. Otherwise the color returns on cooling. The iodine is also removed from the compound by caustic potash, argentic nitrate, mercuric chloride, and in fact, any chemical for which it has a strong affinity.

Uses.—The principal use of starch is, of course, for food. It serves also as a raw material in the manufacture of alcohol, and is prepared pure on an enormous scale, partly for food, partly for stiffening linen, paper, etc., and also for the preparation of dextro-glucose and dextrin.

LABORATORY EXPERIMENTS.

CELLULOSE AND STARCH.

I. **Preparation of Starch and Cellulose from Potato.**—Grate a potato to a pulp with the tin grater on to the agateware plate. Wash the pulp through a strainer into the agate cup with water from the wash bottle, working it thoroughly at the same time with the fingers. Continue this until the water no longer runs through milky. Put the washed pulp aside on filter paper = *Cellulose, with some starch.* Decant off the water in the cup, carefully saving the whitish sediment in the bottom. Fill the cup again with water, let it settle a few minutes, and decant again with care. Then scrape the white residue on to a piece of filter paper = *Starch.*

II. **Microscopic Examination.**—Examine with both high and low powers a thin section of potato. Notice how the starch granules lie inside the cells whose walls are of cellulose. Then add a drop of iodine solution diluted three or four times with water, and examine again. Notice the *blue* starch granules. Examine, both with and without iodine, some of the prepared starch and some corn starch, mixing a very little of each with a drop of water and spreading it well out on the slide. Notice the

"oyster-shell" markings of the potato starch, and the star-shaped "hilum" of the corn starch.

III. **Chemical Tests.**—1st. Fill a test-tube half full of potassic hydrate (KOH), and add a little starch in powder. The starch swells and forms a paste in the cold.

2d. Fill the agate saucepan half full of water, place it on a tripod, and boil it vigorously with a Bunsen burner. Put about a tablespoonful of starch into a mortar, and grind it and mix it with enough water to form a smooth thin milk. Add this milk to the water as it boils. Notice that the white color at once disappears and it forms *starch paste.*

Pour some of this paste into a beaker and add a few drops of iodine solution = deep blue. Put this blue paste into four different test-tubes and test as follows:

(*a*) Heat the 1st gently over the flame; the color finally disappears completely. Then let it cool. If the paste was heated till the iodine volatilized, no color will reappear, but if carefully managed the blue color will return when the paste cools.

(*b*) To the 2d add a little KOH; the color disappears. Then add a little dilute hydrochloric acid (HCl dil); the color returns.

(*c*) To the 3d add a few drops of argentic nitrate ($AgNO_3$); the color disappears.

(*d*) To the 4th add some mercuric chloride ($HgCl_2$); the color disappears.

3d. Put a little paste in a beaker; add plenty of water, stir, and filter the mixture into a test-tube. To this clear filtrate add a few drops of iodine = blue, just like the unfiltered paste.

IV. **Cellulose.**—Examine under the microscope, both with and without iodine, some cellulose from I., and also a few shreds of filter paper, thoroughly wet with water. Notice that the iodine hardly stains the cellulose at all, while it turns blue any grains of starch that may be present.

If you have the time, examine some filter paper and grains of starch under the microscope, with the aid of polarized light.

Place a few shreds of filter paper, wet with water, in three different test-tubes, and test as follows:

(*a*) To the 1st add some water and boil; this has no effect.

(*b*) To the 2d add some KOH ; the fibres swell slightly, and, after a time, become more or less gelatinous.

(*c*) To the 3d add a few drops of common sulphuric acid* (H_2SO_4); the cellulose turns brown or black and quickly dissolves.

(*d*) Put some cellulose from I., and some filter paper, into separate test-tubes, and add to each an inch or so of the Cupric Hydrate solution. Shake, warm gently, and notice that the cellulose dissolves completely.

Put this solution of cellulose in a small beaker and add some dilute hydrochloric acid, stirring it gently, until the deep blue color disappears. Notice that the cellulose is reprecipitated in a stringy, amorphous condition.

* This acid is especially corrosive; it should be used carefully, and carefully replaced on the shelf.



FIG. 1.—POTATO STARCH. × 160. Without and with polarized light (Dr. E. G. Love).

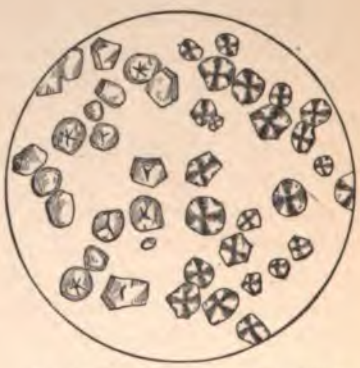


FIG. 2.—CORN STARCH. × 320. Without and with polarized light (Dr. E. G. Love).

(*b*) To the 2d add some KOH ; the fibres swell slightly, and, after a time, become more or less gelatinous.

(*c*) To the 3d add a few drops of common sulphuric acid* (H_2SO_4); the cellulose turns brown or black and quickly dissolves.

(*d*) Put some cellulose from I., and some filter paper, into separate test-tubes, and add to each an inch or so of the Cupric Hydrate solution. Shake, warm gently, and notice that the cellulose dissolves completely.

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FIG. 1.—POTATO STARCH. × 160. Without and with polarized light (Dr. E. G. Love).

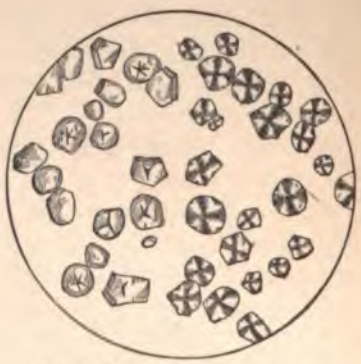


FIG. 2.—CORN STARCH. × 330. Without and with polarized light (Dr. E. G. Love).

LESSON II.

DEXTRIN, GLYCOGEN AND GLUCOSE.

THE DEXTRINS.—$(C_6H_{10}O_5)_n$.

In the process of conversion of starch or of cellulose into the simpler compounds, maltose or glucose, by the action of either heat, chemical agents, or ferments, a series of intermediate products are formed, known as dextrins. Some authorities claim that the different individuals of this group can be accurately separated and recognized by their behavior with polarized light, the colors they give with iodine solutions, and other properties. They have even given names to them, in some cases simply calling them α, β, and γ dextrins; in other cases naming them after their reaction with iodine, as Achroo (colorless) or Erythro (red) dextrins; or, according to their source and general characteristics, as Amylo (starch) or Malto dextrins. In the opinion of others the dextrins are simply mixtures of granulose or soluble starch with varying proportions of maltose or glucose.

Occurrence and Preparation.— Dextrin, in one variety or another, occurs to some extent in nature associated with cane sugar and the glucoses. It is prepared pure on a large scale either by heating finely powdered dry starch, with constant stirring, until it gets brown, a temperature of from 225°–260° C. being the best for this purpose; or else by first soaking the starch in very dilute nitric acid (1 or 2%) and then drying it thoroughly at a temperature of 100° or 110° C. It is also formed as an intermediary product in the digestion of starchy foods, in the manufacture of beer and alcohol, and in the conversion of starch into dextro-glucose or corn sugar. In the latter case it enters very largely into the finished product in the form of syrups or liquid glucose.

Properties.—All of the dextrins are easily soluble in water,

making a clear, sticky solution; they dissolve somewhat in dilute but are insoluble in absolute alcohol. They are structureless, and more or less sweet to the taste. They rotate the ray of polarized light to the right, the specific angle of rotation for the yellow sodium ray, known as $[\alpha]_D$, being from $+ 170^\circ$ to $+ 195^\circ$. This angle, in every case, is the amount of rotation produced by a column 1 decimetre long of a solution of 1 gramme of the substance in 1 cubic centimetre of a non-active solvent.

With diluted iodine solution they give various shades of color, from the purplish blue of the amylo-dextrin, which is hardly to be distinguished from the granulose of starch, through the brown and red of the erythro-dextrins, to the almost colorless compounds made with the malto-and achroo-dextrins, which are much like maltose and dextrose. They are easily broken down to maltose or glucose by boiling with water or, more rapidly, with dilute acids; by the action of stronger acids, and also of the various amylolytic ferments, such as ptyalin, amylopsin, diastase, and others. When pure, they do not undergo alcoholic fermentation, but in the presence of some fermentible carbohydrate, they ferment readily and thoroughly. This is probably due to the secretion by the yeast plant of a special ferment, which acts like diastase and the others in reducing the dextrin to maltose.

Uses.—Dextrin is more or less valuable for food; toast, the crust of bread, and the different kinds of prepared infants' foods, are common examples of its use. It has the advantage over starch of already being partially digested. It also enters into the manufacture of beer and alcohol. It is very largely used as a substitute for the different gums, especially gum arabic which it closely resembles, in the preparation of mucilage, in dyeing and calico printing, and in the finishing and glazing of cards, wall paper, and similar articles.

GLYCOGEN.—$C_6H_{10}O_5$, or perhaps $C_{36}H_{62}O_{31}$.

Although the experiments on this substance will be made in a later lesson, it is proper to briefly describe it before leaving this class of the carbohydrates.

Occurrence.—Glycogen, or liver dextrin, as it is sometimes called, is the peculiar carbohydrate found in the livers of all

animals, and also, in smaller quantities, in the muscles and other organs, especially in fœtal life. It occurs, in some instances in considerable abundance, in certain shell-fish, such as mussels and oysters. It has also been found in the vegetable kingdom, principally in some of the varieties of fungi and other low orders of plants, *e. g.*, in the common truffle, in Mucor Mucedo and other mould plants, and perhaps in the yeast plant.

Preparation.—It is best prepared from the livers of rabbits or dogs that have been well fed, for some days, upon a carbohydrate diet. Directly after the animal is killed the liver must be taken out, cut into small pieces and plunged into boiling water, to prevent the glycogen fermenting into glucose. The pieces are then hashed or ground up as fine as possible, stewed in the same water for some minutes, strained, and then the proteids separated by, among other reagents, hydrochloric acid and mercury potassium iodide. The glycogen is then precipitated from the aqueous solution by an excess of alcohol.

Properties.—It is claimed by some experimenters that, by variations in feeding, different varieties of glycogen can be obtained. This is probably, however, due to the presence of impurities derived from the liver.

Glycogen, as we meet it, is quite similar to the higher (*i. e.*, starch-like) members of the dextrin group, and, like them, can be readily changed to other dextrins and to maltose and glucose, by heat, dilute acids, and ferments. In fact, our theories with regard to this substance demand the presence of two ferments in the liver, the one to form glycogen from the maltose and glucose of the portal vein, and the other, which is present after death and acts quite rapidly, to change it into glucose again as needed by the system.

It is a white, amorphous powder, easily soluble in hot water, and has the following characteristic reactions:

1st. Its solutions are opalescent, not clear. They can be cleared by potash or acetic acid.

2d. Its solutions are colored red or brown by iodine, the color disappearing, as in the case of starch, on heating or on the addition of an alkali or various metallic salts.

3d. Its solutions polarize strongly to the right, $[\alpha]_D$ being about $+211°$.

4th. Its solutions are precipitated by alcohol.

Uses.—Glycogen seems to be of the utmost importance in physiology, in enabling the liver to act as a reservoir of carbohydrate food. During digestion an excess of sugar is poured into the system through the portal circulation, but instead of going at once all over the body it is changed to glycogen in the liver, and given out slowly and regularly from there into the general circulation. Glycogen is also formed in the liver by both proteids and fats. During a period of fasting the store of glycogen in the liver is probably the first to be called upon. It is supposed to bear an intimate relation with certain morbid conditions of the body, such as diabetes mellitus, but this has not yet been accurately determined.

THE MONO-SACCHARIDS OR GLUCOSES.—$C_6H_{12}O_6$.

Leaving to the next lesson the class of di-saccharids or sugars proper, we come now to the class of mono-saccharids or glucoses. These are the simplest in structure of all the carbohydrates, and are produced by the breaking down of the higher members.

DEXTRO-GLUCOSE OR DEXTROSE.

(*Grape Sugar*) (*Corn Sugar.*)

This substance is so much better known and more widely disseminated than any of the other members of the class that in common language it has practically monopolized the word glucose. Its name dextrose is derived from its property of "polarizing" to the right.

Occurrence.—It is found widely scattered in the vegetable kingdom, generally associated with an equal quantity of lævulose, and forming the so-called Invert Sugar. It occurs largely in sweet fruits, grapes, plums, figs, etc.; and the sugar that crystallizes out of these fruits when dried, as in raisins, for instance, is either dextrose or a loose combination of dextrose and lævulose. It also occurs in the juices from every part of plants, and is present in considerable quantities in honey, though whether coming directly from the nectar of flowers, or produced by fermentation from

cane sugar or other carbohydrates, it is hard to say. It is found in raw sugar, both from the beet and the sugar cane, in molasses, in the peculiar manna sugar, in crude gums, and, in fact, in almost every vegetable extract.

In the animal kingdom it seems to be the form in which the carbohydrates are absorbed into the circulation. Accordingly we find traces of it in the blood, especially in that of the portal vein. It also seems to occur in hens' eggs, and in the liver, where it is probably derived from glycogen. It must be remembered in this connection that it is impossible, in small quantities, to distinguish it from maltose or from lactose, either of which may be equally present. There is no doubt, however, that it occurs, often in large quantities, in the urine of patients suffering from diabetes mellitus, and forms the essential feature of the disease.

Preparation.—Dextrose is formed in nature by the breaking down of cane sugar, or in some cases of maltose or glycogen.

It is manufactured on a large scale by heating starch or cellulose with dilute acid, as illustrated in Lesson III.

Properties.—Dextrose occurs in commerce in two forms, as anhydrous dextrose ($C_6H_{12}O_6$) and as the hydrate ($C_6H_{12}O_6 + H_2O$). The latter is formed when dextrose is allowed to crystallize in the cold from a water solution; the former, when the solution is heated. Both these forms are crystalline, and by careful heating the latter loses its water and becomes anhydrous without changing its form. Both varieties polarize to the right, $[\alpha]_D$ for a solution of the hydrate being $+48.2°$, and for the anhydrous dextrose $+53.1°$. Dextrose is not more than one-half as sweet as cane sugar. It is very soluble in water, easily soluble in dilute and slightly in absolute alcohol. It diffuses easily. With yeast it readily ferments to alcohol and carbon dioxide, but other microbes produce in it sometimes alcoholic, frequently lactic, butyric, and other fermentations.

Uses.—Dextrose is principally used as a food, either in the form of fruits, molasses, honey, etc., or in a more pure form. Dry dextrose is sometimes employed as an adulterant of cane sugar, this depending largely on the relative price of the two, and is much used to take the place of cane sugar in confectionery. The dextrose syrups are largely used instead of molasses, and also in

the production of artificial honey, the adulteration of beer, preserving fruits, and strengthening wines and vinegars. Some dextrose is also used in the manufacture of printers' rollers, of copying inks, and of caramel or sugar color.

LÆVULOSE.—$C_6H_{12}O_6$.

Occurrence.—This glucose is found associated with dextrose and cane sugar in sweet fruits, molasses, raw sugar, honey, and in the various vegetable juices. It is usually in the combination with dextrose known as invert or fruit sugar, resulting from the hydration of cane sugar.

Preparation.—Lævulose can be prepared in a pure form only with very great difficulty, either from invert sugar or from certain carbohydrates such as inulin, which, on breaking down, produce lævulose just as starch produces dextrose.

Properties.—It is possible to obtain it in the form of fine needle-shaped crystals. Ordinarily on evaporation it forms a thick syrup, which not only refuses to crystallize itself, but has the property of keeping in solution considerable quantities of dextrose and cane sugar, which would otherwise crystallize out. For this reason the formation of invert sugar in the process of refining cane sugar is guarded against as much as possible.

The name lævulose was given to it from its property of polarizing strongly to the left, $[a]_D$ for a solution of the pure crystals at 20° C. being equal to $-71.4°$. In other respects it strongly resembles dextrose, responding to the same tests and fermenting in the same manner.

GALACTOSE.—$C_6H_{12}O_6$.

This glucose is formed, along with dextrose, by the breaking down of lactose and also of some rarer carbohydrates by boiling with dilute acids, or by the action of certain ferments. It crystallizes in small six-sided crystals; it polarizes to the right more strongly than dextrose, $[a]_D$, at 20° C., being equal to 80° it responds to all the glucose reactions, and it undergoes alcoholic fermentation more or less readily. Its chief interest to us is in connection with the manufacture of koumyss.

TESTS FOR GLUCOSE.

The more important tests for glucose may be divided up into five principal classes, according to whether they depend upon—

(*a*) *The action of alkalies.*—Moore's test.

(*b*) *The reaction with hydrazin compounds.*—Phenyl hydrazin test.

(*c*) *The reduction of metallic and other compounds.*—Bismuth subnitrate, picric acid, Trommer's and Fehling's tests.

(*d*) *Alcoholic fermentation.*

(*e*) *The polariscope.*

Taking these up in the above order we have—

1st. *Moore's Test.*—When glucose, either dry or in solution, is heated with a powerful alkali, it rapidly decomposes into a variety of different substances, some of which—acetol, acetone, and others—are light volatile liquids, others of which are the ordinary formic, acetic, and lactic acids, and still others are brown-colored amorphous bodies, as yet little investigated, but described as compounds of humic and other acids. The latter give the mixture a decided yellow or brown color, which, on acidifying with nitric acid, disappears more or less completely, leaving behind a peculiar odor of caramel.

On this reaction is based the simplest of all the glucose tests, where the suspected liquid is heated to boiling, with the addition of caustic alkali. It is best, in practice, to heat only the top part of the mixture, so that any change of color is at once noticed; this is especially important when dealing with samples of urine, which generally have a decided yellow or brown color at the start. It is always advisable to confirm the result with nitric acid.

The test is not a very delicate one, and is rather unreliable in testing urines, not only because the mixture is colored more or less deeply to start with, but also because the same reaction sometimes occurs with normal constituents of the urine, notably with mucin.

2d. *Phenyl Hydrazin Test.*—This test, which was introduced by Fischer in 1884, depends on the formation of a compound known

as Phenyl Glucosazon, by the action of glucose on an excess of phenyl hydrazin ($C_6H_5.NH.NH_2$).

When dilute solutions of these two substances are mixed together in the presence of acetic acid, and heated, the following reaction takes place :

$$C_6H_{12}O_6 + 2C_6H_5N_2H_3 = C_6H_{10}O_4(C_6H_5N_2H)_2 + 2H_2O + H_2$$

Dextrose. Pheayl Hydrazin. Phenyl Dextrosazon.

The hydrogen set free in this reaction does not escape, but attacks some phenyl hydrazin, changing it to anilin and ammonia.

The phenyl dextrosazon, which is precisely similar to phenyl lævulosazon formed from lævulose, and is often called phenyl glucosazon, consists of yellow needle-shaped crystals, which melt at 204 or 205° C., are insoluble in boiling alcohol, dissolve only slightly in water, reduce Fehling's solution, and polarize slightly to the left. They are readily recognized by their appearance under the microscope, and are distinguished by that and by their melting point from similar compounds with other carbohydrates. This test is an extremely delicate and an extremely accurate one, giving reliable results where other tests fail.

3d. *Bismuth Subnitrate Test.*—This and the following tests depend upon the reduction or deoxidation of various compounds, both organic and inorganic, in alkaline solutions, by the action of glucose. As is seen by the formula $C_6H_{12}O_6$, there is only enough oxygen present in glucose to burn up the hydrogen, and consequently it will, under favorable circumstances, act as a powerful reducing agent, *i.e.*, take oxygen away from other bodies.

In the present test bismuth subnitrate, $Bi(OH)_2NO_3$, is reduced to bismuth suboxide, Bi_2O_2, which forms a characteristic cloudy black precipitate. The original method of making the test was to mix with the suspected liquid an equal bulk of carbonate of soda solution and a pinch of dry subnitrate, and then to boil it. It is more satisfactory to use Nylander's modification of this test, when the bismuth salt is dissolved in an alkaline solution of Rochelle salt. The Nylander's solution is composed of 4 parts Rochelle salt, 10 parts caustic soda, and 2 parts bismuth subnitrate, in 100 parts of water. The soda in this makes the solution alkaline, and the Rochelle salt keeps the bismuth dissolved.

This test is not so delicate as some of the others, and has the serious disadvantage of reacting with other substances, sulphur (from albumin) and various reducing compounds, which not uncommonly occur in urine.

4th. *Picric Acid and Potash Test.*—When a solution containing glucose is boiled with an alkaline solution of picric acid or trinitrophenol, $C_6H_2.(NO_2)_3.OH$, the latter is reduced to picramic acid, $C_6H_2(NO_2)_2.NH_2.OH$, with the production of a deep brown or even black color. This reaction must not, however, be mistaken for the slight change in color which results from boiling picric acid and potash together by themselves.

This test is one of the most delicate that we possess; and is specially important to a student of medicine, because picric acid not only reacts for glucose, but also, as is seen later, is an excellent reagent for albumin. Kreatinin and some similar compounds, and acetone, which also may occur at times in urine, respond to this test in the same manner as glucose.

5th. *Trommer's Test.*—This test, as well as Fehling's test, which follows, depends upon the reduction by glucose of an alkaline solution of cupric hydrate, $Cu(OH)_2$, into the yellow cuprous hydrate, $Cu_2(OH)_2$, and finally, by continued boiling, into red cuprous oxide, Cu_2O.

In Trommer's test an excess of potash is first mixed with the suspected fluid, and afterwards cupric sulphate is slowly added, producing at once cupric hydrate. The latter is a bluish-white precipitate, insoluble in potash, but dissolving in a solution of glucose. Hence if any glucose is present a deep blue solution results. To obtain the best results the mixture must be saturated with the $Cu(OH)_2$. The characteristic yellow and red cuprous salts result slowly in the cold, but almost instantaneously on heating.

Both this test and Fehling's test are delicate and useful, but they respond to many substances, uric acid, kreatinin and others, which often occur even in normal urines.

6th. *Fehling's Test.*—This test is like Trommer's test excepting that Rochelle salt, and not glucose, is depended on to keep the cupric hydrate in solution. Fehling's solution consists of a mixture of cupric sulphate, Rochelle salt, and caustic alkali;

but as this mixture is constantly liable to decompose, especially when exposed to light, it is far preferable to keep the cupric sulphate in one solution and the Rochelle salt and alkali in another. These solutions are made of the following strength:

500 c.c. of the cupric sulphate solution contain 34.64 grammes of crystallized $CuSO_4$.

500 c.c. of the Rochelle and soda solution contain— 187 grammes of Rochelle salt. 68 " of sodic hydrate.

Fehling's solution proper is made by mixing together equal quantities of these two solutions.

To make a qualitative test it is only necessary to mix a few drops of the two solutions, dilute more or less with water, boil thoroughly, and add a little of the suspected fluid to the boiling mixture. It is always advisable to boil the solution thoroughly before making the test, as a safeguard against error. To make the test more delicate it is only necessary to use a more diluted Fehling's solution. When carefully made, the test is extremely delicate. It fails, however, now and then when dealing with impure and putrid solutions, such as stale pathogenic urines.

Quantitative Determination of Glucose. *Fehling's Test.*—To use the Fehling's solution for a quantitative test, advantage is taken of the fact that a certain amount of glucose is always necessary to decompose any given amount of cupric sulphate, and that when the cupric sulphate is all decomposed the solution loses its blue color and becomes colorless. The copper solution is made of such a strength that 5 c.c. of it are exactly decomposed by 0.05 gram of dry glucose. Hence, whenever, in making the test, we add enough of the liquid we are testing to destroy the blue color in 5 c.c. of the copper solution, we know that we have added at the same time, dissolved in that liquid, 0.05 gm. of glucose. If we have to add 1 c.c. of the liquid before the color disappears, then there is 0.05 gm. glucose in 1 c.c. of the liquid, or 5 gms. in 100 c.c.; in other words, the liquid will contain 5% of glucose.* If 2 c.c. are needed before the color disappears the liquid is only half as strong, and contains only 2½%. If it takes 5 c.c. the strength of the liquid is only 1%. Hence, in general,

* The correction for difference of specific gravity is too small to be taken into consideration.

the percentage of glucose in the liquid to be tested will be always equal to 5, divided by the number of c.c. of liquid used.

This Fehling's test can, with care, be made with considerable accuracy. The principal error is in not knowing just when to stop adding from the burette. The solutions, burette, pipette, flask, etc., should all be kept scrupulously clean; and the Fehling's solution, diluted, and with a piece or two of pumicestone in it to prevent bumping, should be thoroughly boiled both before and during the addition of each drop of the test liquid. The latter should be added at first drop by drop, and only when the reaction has satisfactorily started can it be run in at all rapidly. The precipitated cuprous compounds should appear first of a purplish color, then, as more and more of the copper salt is decomposed, they get more and more red, till they finally give the liquid a bright vermilion color. That marks the end of the reaction.

To make sure of not adding too much glucose solution, the precipitate should be allowed to settle now and then, and the color of the supernatant liquid observed while still hot. If allowed to cool, some of the cuprous salt will oxidize again. The liquid should be practically colorless. If bluish it shows that some of the copper is still unreduced, and more test liquid is needed. If too much glucose, however, has been added, the liquid has a yellowish or even brownish look from the action of the alkali on the glucose. Now and then, in spite of every precaution, the solution turns a muddy greenish color directly the test liquid is added, and then often no subsequent treatment will save the test. This is especially the case in urine analysis, and is the chief objection that can be raised against this method of analysis.

The subject of alcoholic fermentation, both by itself and as a test for glucose, will be discussed later.

Before leaving this topic, a word should be said about the polariscope method of testing for glucose. The polariscope is is an instrument for determining the exact angle of rotation of any particular solution. If this is found, and if we know the length of the test column and the specific angle of rotation of the substance tested for, we can easily calculate the strength of the solution. For scientific and commercial analyses of carbo-

hydrates, and especially of cane sugar, this is by far the most accurate and most convenient method; but in medical practice it is of but little value as compared to the tests described above, partly because it needs an expensive and more or less complicated piece of apparatus, but principally because, before using it, the liquids to be tested have to undergo a tedious and elaborate system of decoloration. Another serious disadvantage is the frequent presence in urine of various organic substances which have a decided and often a reverse action on polarized light.

LABORATORY EXPERIMENTS.

DEXTRIN AND GLUCOSE.

I. **Dextrin.**—Two samples. Test each sample as follows:

(*a*) Taste it.

(*b*) Dissolve it in water; notice that it is sticky and gummy.

(*c*) Half fill a test-tube with alcohol, and add one drop of dextrin solution = a white precipitate (ppt.). Add some water; the ppt. dissolves.

(*d*) Place some dextrin solution in a test-tube and add some iodine solution diluted three or four times with water. Notice the red "claret" color with the one dextrin and the brown color with the other.

II. **Glucose.**—QUALITATIVE TESTS.—Test glucose as follows:

(*a*) Taste it.

(*b*) Dissolve it in water. On this solution make the following tests:

1st. *Moore's Test.*—Put some solution in a test-tube and add one-third as much KOH. Boil the upper layer of the solution = *brown color*. Notice the smell of caramel. Add a little concentrated nitric acid (HNO_3 conc.) and the color will disappear.

2d. *Phenyl Hydrazin Test.*—Measure out with the tip of a penknife blade two parts of phenyl hydrazin (hydrochlorate) and three parts of acetate of soda, and put them in a small test-tube. Fill the tube two-thirds full of glucose solution, dissolve the

salts by warming them gently and by agitation, and then stand the test-tube in boiling water in a waterbath for twenty or thirty minutes. After this take it out and put it into a beaker of cold water. Notice, as it cools, the formation of a yellow crystalline ppt. of phenyl glucosazon. Let the ppt. settle, especially if the solution is very dilute, and examine it under the high and low powers of the microscope, noticing the radiating groups of yellow needle-shaped crystals.

3d. *Bismuth Subnitrate Test (Nylander's Test).*—Put in a test-tube some glucose solution and boil. To the boiling liquid add half an inch of Nylander's solution. Boil for two or three minutes more and let it stand. Notice the black ppt., which appears at once if the glucose solution is strong, and more slowly if it is dilute.

4th. *Picric Acid and Potash Test.*—Put some water in a test-tube, add a few drops of picric acid and half an inch of KOH and boil. Notice that the color changes somewhat, although no sugar is present.

In another test-tube put a little glucose solution, add the picric acid and KOH, and boil. Notice that the color changes at once to very dark brown or black.

5th. *Trommer's Test.*—Put some of the glucose solution in a test-tube and add about one-fourth its volume of KOH. Then add the cupric sulphate ($CuSO_4$) drop by drop, shaking constantly, until the bluish-white ppt. of cupric hydrate, $Cu(OH)_2$, just ceases to dissolve and makes the liquid slightly turbid. Set the mixture aside, let it stand, without warming, and notice that a yellow ppt. slowly forms in the course of fifteen or twenty minutes.

Repeat the test in another test-tube, and boil the mixture. It will give a red or yellow ppt. at once.

6th. *Fehling's Test.*—Mix equal parts of $CuSO_4$ and of "rochelle and soda" solution together in a test-tube. The deep blue mixture is *Fehling's solution.* Put a few drops of it in a test-tube, add a little water, and boil. To the boiling liquid add a drop or two of the glucose solution, and keep it boiling. It will give a yellow and then a red ppt.

Repeat these five tests with more and more dilute glucose solutions, observing the comparative delicacy of each test. Also,

if you have time, examine both grape juice and raisins for glucose with the test you prefer.

QUANTITATIVE DETERMINATION OF GLUCOSE. — *Fehling's Method.*—Take with a pipette 5 cubic centimetres (c.c.) of the $CuSO_4$ and place in the flask. Rinse the pipette, then take with it 5 c.c. of the "rochelle and soda" solution and add them to the same flask. Fill the flask one-third full of water, add a piece of pumice stone, and boil. While this is heating, fill the burette to the zero mark with the "test glucose solution"; and when the copper mixture is boiling, add to it some of this solution, drop by drop, from the burette. Every minute or two stop boiling, let the ppt. (which should be red) settle, and notice if the blue color has disappeared from the liquid in the flask. If it has not, boil again, add a few drops more of the glucose solution, and examine the color.

When the blue color has entirely disappeared, read on the burette the number of c.c. used, and calculate the percentage of glucose in the solution by the following rule:

"The percentage of glucose in the solution is equivalent to 5 divided by the number of c.c. of solution used."

Repeat this experiment with a dilute solution of the solid glucose.



LESSON III.

CONVERSION OF STARCH INTO DEXTRIN, GLUCOSE, AND MALTOSE. FERMENTATION.

THE CONVERSION OF STARCH INTO DEXTRIN, GLUCOSE, AND MALTOSE.

The following experiments illustrate the more important methods of breaking down starch into the simpler carbohydrates.

Preparation of Dextrin.—In Section I. is shown one of the ordinary ways of preparing dextrin on a large scale. The starch granules begin at from 150° to 160° C. to lose their characteristic structure, to get yellow and even brown in color, and to become more or less completely soluble in water. After keeping at this temperature for some time, the conversion is almost complete. The addition of even a trace of nitric acid assists greatly the formation of the erythro-dextrins.

In commerce, this heated starch is generally extracted with water, and the solution evaporated and heated a second time. In the laboratory much of the dextrin produced is amylo-dextrin, which greatly resembles and gives the same color with iodine as the soluble starch of Lesson I.

In order to recognize the presence of the erythro-dextrins, it is often necessary to make the dextrin solution very strong and the iodine solution very weak, and, if the resulting color is purplish blue, to add more dextrin solution to the mixture till the blue just disappears, when the red or brownish color may be seen.

Manufacture of Glucose.—In Section II. is illustrated the regular method of manufacturing commercial glucose or corn sugar. The pure starch which is prepared in large quantities, usually from Indian corn, is boiled, often under pressure, with diluted acid. Sulphuric and oxalic acids are the ones most commonly employed, because they can be most easily and

cheaply removed from the solution afterward. When the conversion has proceeded far enough—a question determined in practice, as in our lesson, by noticing the color given by iodine—the acid is neutralized and precipitated by the addition of either limestone, $CaCO_3$, or, in our case, of barium carbonate. The acid combines with this, forming calcium or barium sulphate, and liberating carbon dioxide, according to the formula,

$$H_2SO_4 + BaCO_3 = BaSO_4 + CO_2 + H_2O.$$

This sulphate is removed by either settling or filtration, and, on evaporating the clear solution, the dextrose will crystallize out. Where syrup is desired, and not dry glucose, the conversion is stopped while some dextrin still remains, and the solution is not concentrated so far.

The Action of Ferments on Starch.—Sections III. and IV. illustrate the action upon starch of two of the most important amylolytic ferments, diastase and ptyalin. These ferments have the power of changing starch into dextrin and maltose, and finally into dextro-glucose.

The ferment known as diastase is found in all kinds of grain when sprouting, although it is most commonly obtained from malt, *i. e.*, barley allowed so sprout a little, and then dried at a temperature high enough to kill the plant but not destroy the ferment. The diastase seems to be formed from the albuminoids around the embryo just when the young plant commences to grow, and it plays, as mentioned before, a most important part in digesting the starch previously stored up in the seed or tuber. There is enough diastase, however, in the malt, not only to digest the starch in the grains themselves, but also to convert many times their bulk of fresh starch.

The ptyalin ferment, existing in the saliva, plays rather an unimportant part in the digestion of the carbohydrates. It acts in the same manner as diastase, only not so powerfully, being unable to attack uncooked starch grains. It acts best at a somewhat lower temperature than diastase, at about 40° C. as compared to 50° or 55° C., and is nearly inactive at the temperatures 60°–65° C., at which, in practice, malt is usually employed. Both act best in neutral rather than alkaline solutions; their action is stopped by

free acids even in small quantities, they are killed by boiling, and are seriously affected by various toxic agents.

FERMENTATION.

Before leaving this subject, it is worth while to discuss briefly the properties and actions of some of the more important ferments that we shall meet in the course of these lessons.

The subject of fermentation has been an important one from the early ages of chemistry. The name was derived from the boiling or bubbling of the carbon dioxide, early noticed in the production of alcohol, and was given at first to almost any process of nature involving change of form or substance which could not be readily explained. Fermentation was the name which covered not only all kinds of decomposition and putrefaction, but also the formation of blood and secretions, the production of heat and cold in the body, and even all kinds of disease. Toward the end of the last century the different kinds of fermentation proper were more carefully studied, and the discussion as to the true nature of alcoholic fermentation began then, only to be finally settled in comparatively recent times.

CLASSIFICATION OF FERMENTS IN GENERAL.—At present we may define a ferment as a chemically active body, a small amount of which transforms large quantities of other substances without itself *apparently* contributing anything to the reaction.

Under this definition we may divide ferments up into three general groups—

1st. *Bodies acting like ferments.*

2d. *Unorganized ferments, enzymes.*

3d. *Organized ferments.*

BODIES ACTING LIKE FERMENTS.

The substances that are included in this first class have to do with several very important chemical reactions, which were long unexplained.

One of the most prominent of them is the substance nitrogen peroxide, NO_2, which, with the accompanying N_2O_3, plays such an important part in the manufacture of sulphuric acid. As

will be remembered, in order to oxidize SO_2, sulphurous anhydride, into SO_3, sulphuric anhydride, the nitrous fumes, as they are called, are necessary to act as carriers of oxygen from the atmosphere to the sulphur vapors. These fumes, however, are not destroyed in the operation: they give up oxygen to the SO_2, and take it again from the atmosphere, and at the end of the process there is, theoretically, as much of the nitrogen peroxide as there was to start with, although immense quantities of sulphuric acid may have been manufactured in the mean time. In other words, the nitrous fumes have played the part of an unorganized ferment.

Another example is the use of sulphuric acid in the manufacture of ethylic or sulphuric ether.

Another is the action of salts of copper in the so-called Deacon's chlorine process.

These reactions, however, can all be explained by imagining an intermediate reaction, in which the active agents take part, only to be set free again in a later stage.

UNORGANIZED FERMENTS.

It is possible that the unorganized ferments or enzymes do their work in some similar manner. These substances are all amorphous nitrogenous bodies, readily soluble in water and generally precipitated by alcohol. They occur in both animals and plants and seem to be secreted by cells in the tissue where they belong. Probably in all instances, certainly in many, as in the case of pepsin, rennet, and others, these cells secrete a zymogen, *i.e.*, a substance inactive of itself, but which, in the presence of HCl or other media and under suitable circumstances, is changed into the ferment.

They are prepared, generally, by extracting them from their source by means of water, salt solutions, diluted acid (especially hydrochloric), and, best of all, by glycerin. It is difficult to obtain them in a pure form; they are rarely free from proteids and from mineral matters. So far as we can tell, though, their composition is much the same as that of albumin, with, generally, less carbon and more oxygen. It is noteworthy that, while of

extremely complicated structure, they are often much less liable than the proteids to undergo decomposition.

Their action depends very largely upon external conditions such as temperature, reaction, presence or absence of certain chemicals, etc. With regard to temperature, each ferment has its limits within which it acts, its temperature of maximum efficiency and its death temperature. They stand dry heat much better than moist heat. It is possible that their destruction in the latter case is caused by coagulation.

CLASSIFICATION.—The unorganized ferments that have been so far isolated may be classified as follows:

I. *Inverting ferments.*—Invertin.

II. *Saccharifying* (diastatic, amylolytic).—Diastase, ptyalin, amylopsin.

III. *Glucoside decomposing.*—Emulsin, myrosin.

IV. *Peptonizing.*—Pepsin, trypsin, papaïn.

V. *Coagulating.*—Rennet, fibrin and myosin ferments.

VI. *Fat-decomposing.*—Steapsin.

Inverting Ferments.—These have the power of inverting cane sugar, *i.e.*, of changing it to dextrose and lævulose. Ferments of this sort have been found in the gastric and intestinal juices, from which it is supposed that cane sugar is changed to glucose in the process of digestion. The most important ferment of this class, invertin, is extracted by water from yeast, either dead or alive, and, from its relations to the theories of fermentation, has been studied very carefully. It enables yeast to ferment, although slowly and indirectly, a solution of pure cane sugar.

Saccharifying Ferments. — These have already been somewhat discussed. They decompose starch, erythro-dextrin, and glycogen into maltose and achroo-dextrin, and will, on further standing, decompose some of the maltose into dextrose. The amylopsin, which is derived from the pancreatic juice, is more powerful in its action than ptyalin, which it otherwise very closely resembles. Besides the three important ones already mentioned, ferments of this class have been found in the liver, intestinal juice, red blood cells, and other organs of the body, and in many parts of plants.

Glucoside Decomposing Ferments. — The ferments belong-

ing to this class decompose a peculiar series of vegetable compounds known as glucosides. These glucosides, of which a great number are known, and some of which have been manufactured synthetically, have the property of decomposing, when warmed with dilute acids or when treated with the proper ferment, into a glucose and some other body. The glucose is usually dextrose; but in some cases very peculiar members of the group result, which are obtained in no other way. The best known ferments of this group are emulsin, which is found in sweet and bitter almonds, and myrosin, found in black mustard seeds.

Peptonizing Ferments.—The action of this class of ferments will be discussed later under Lesson XXI. It is only necessary to say here that they are able to break down the proteids and some albuminoids into diffusible compounds. The pepsin, which is obtained from the gastric juice, acts best in a solution of hydrochloric acid, preferably 0.2%, and but partially accomplishes the digestion of the proteids of the food. The work is carried forward by the trypsin of the pancreatic juice, working in an alkaline or neutral medium. The papaïn is a vegetable digestive ferment, which digests proteids in the same way as pepsin, but only works in a slightly alkaline or neutral solution.

Coagulating Ferments. — These will also be discussed later. The rennet ferment, which coagulates the casein of milk, exists in the stomachs of all infant mammalia, for the purpose, it is supposed, of making light and easily digested coagula of the milk ingested. The true nature of the fibrin and myosin ferments is still to be determined.

About the steapsin of the pancreatic juice, mentioned under the sixth class, but little is as yet known. For although several experimenters have observed that fat is broken up in digestion into fatty acid and glycerin, the ferment proper has not been isolated, nor is it even agreed that such decomposition is necessary for the digestion of fat.

ORGANIZED FERMENTS.

The ferments discussed so far have all been true chemical substances, producing their effects by the agency of what we now believe to be purely chemical means. There remains, however, the most important group of all, namely, the Organized Ferments, living organisms, which feed upon the substances they decompose, and change them either in their own cells, or at any rate as a part of their life process. These microscopic beings, which are now generally grouped together under the name microbes, are divided into three classes—the mould plants, yeast plants, and bacteria.

Mould Plants.—These are microscopic fungi, growing and propagating like the larger members of the family, *i.e.*, the ordinary mushrooms, puffballs, and toadstools. The growth, and hence the work of these plants is done by the root, or mycelium, consisting of long, slender, white, branching fibres, which under the microscope are composed of long cylindrical cells, rather irregular in shape, adhering one to the other. This mycelium honeycombs the material attacked if a solid, or forms white floating masses through it if a liquid, as long as all conditions are favorable. But when, either from drying up, or failure of food, or any other cause, the active growth is impeded, little white stems appear here and there from the surface of the mycelium; little heads form on these, which gain a characteristic color as they ripen, and which finally burst, scattering the minute spores into the air. To these microbes are due all the phenomena of moulding and much of the ordinary decomposition and decay.

Yeast Plants.—These belong to a much lower order of life. They consist of a single cell, with a cell-wall which consists, it is supposed, of cellulose. They are about the size of one of the cells in a piece of mycelium, *i.e.*, describing them roughly, they can be seen with a two-thirds and studied with a one-sixth objective.

There are several varieties of them, which differ in size, shape, and especially in action from each other. They look under the microscope like white grapes, single and in bunches. They propagate by budding, the buds sometimes adhering to the indi-

vidual cell, sometimes separating. They stain quite readily with fuchsin and some other dyes, showing two or three nuclei inside them. They are the active agents in alcoholic fermentation, and also in many other fermentations of less importance.

Bacteria. — These form the smallest class of microbes, needing a high magnifying power—a one-sixth objective, for example —even to distinguish them. They occur in a variety of shapes, from the round micrococci, through the rod-like bacteria and bacilli, to the curved spirilla and spirochætæ. They propagate by division, *i.e.*, the parent cell enlarges and divides across the middle, making two bacteria, both of which can proceed to multiply in the same manner. Some varieties of them also propagate by forming spores. As we see them in liquids they are in constant motion, due partly to the so-called Brownian movement, partly, it is supposed, to individual efforts. They will be found, in these lessons, in great abundance in all organic fluids which have been exposed to the air or have stood unsterilized for any length of time.

LABORATORY EXPERIMENTS.

CONVERSION OF STARCH INTO DEXTRIN, MALTOSE, AND GLUCOSE.

I. **Into Dextrin by Dry Heat.**—Put half a spoonful of dry powdered starch on the agate plate placed on a sand-bath. Moisten the starch with a solution of one drop of dilute nitric acid, HNO_3 dil., in a small test-tube full of water. Heat it very gradually, stirring the starch constantly with a knife. When it is well browned, dissolve some in water, filter, and test the clear solution as follows:

(*a*) Add a drop of diluted iodine solution; notice the resulting color, usually red or brown, although often blue if too much iodine is added.

(*b*) Fill a test-tube half full of alcohol, and add one drop of the solution. Usually, though not always, a white ppt. appears.

II. **Into Dextrin and Dextro-glucose by Boiling with Acid.**— Fill the agate cup half full of water and heat to boiling. While it is heating, grind up all the remaining starch to a milk with cold water. Add gradually to the boiling water enough of this starch to form a stiff paste, when thoroughly stirred in. Reserve enough of this paste for tests III. and IV. below. To the rest, in the cup, add 10 c.c. of H_2SO_4 dil. and boil for an hour or so. Every few minutes take out a few drops with a pipette and test for dextrin by adding to it a drop or two of much diluted iodine solution. Notice that the resulting color is at first blue, but after more prolonged boiling it changes to red or brown, and finally hardly any color at all is produced. Then neutralize with the milky solution of baric carbonate ($BaCO_3$) till *blue* litmus paper, dipped in the liquid, no longer turns red. Boil up for a minute, then let it settle, and decant or filter the liquid till clear. Test this liquid for glucose by the qualitative glucose tests.

III. **Into Dextrin and Maltose by Malt (Diastase Ferment).**— Put some starch paste from II. into two little evaporating dishes. When nearly cool (not over a blood-heat) add to the first a few crushed grains of malt. To the second add a little extract of malt made by soaking some crushed malt in water and filtering the liquid. Let them stand a few minutes, and then test each for maltose with Fehling's solution.

IV. **Into Dextrin and Maltose by Saliva (Ptyalin Ferment).**— Put starch paste into three clean evaporating dishes. To the first add a few drops of saliva. To the second add a little water solution of saliva, filtered. To the third add a little of the same solution boiled for a moment. Let all three stand some minutes, keeping them a little warm (not over blood heat at any time) by now and then holding them for a minute over the boiling starch solution of II. Then test all three for maltose with Fehling's solution. Maltose will be present in Nos. 1 and 2, but will not appear in No. 3.

LESSON IV.

CANE SUGAR, MILK SUGAR, AND FERMENTATION.

DI-SACCHARIDS OR SACCHAROSES.

This important class of the carbohydrates stands half-way between the starches and dextrins on the one hand, and the glucoses on the other. They possess a definite formula ($C_{12}H_{22}O_{11}$), and at least one of them, maltose, is derived from the breaking down of the more complex bodies. They all readily take up an extra molecule of water, and become converted into two molecules of glucose, the process being commonly called inversion, a name properly given only to the hydrolysis of cane sugar. They differ from the class of poly-saccharids in being crystalline and diffusible. It is harder to distinguish them from the glucoses, but neither cane sugar nor lactose ferment *directly* to alcohol, nor does cane sugar respond to the ordinary glucose reactions already described.

CANE SUGAR.

Saccharose, Sucrose.

Occurrence.—This important substance, by far the most prominent of all the carbohydrates, is found very widely distributed in the vegetable kingdom. It seems to be formed in the leaves, possibly in the same way that starch is, but probably either from starch itself or from derivatives of it, such as maltose or glucose. On a large scale it is obtained either from the juices of certain graminaceæ as the sugar cane and sorghum, or from roots such as the beet, or from the sap of trees like the palm and maple. It occurs also in all sweet fruits, in the nectar and juices of flowers, notably in the case of the cactus plant, and, in small quantities, in honey, manna, and other sweet vegetable products. In the latter instances, owing to the readiness with which it is

inverted by acids as well as by ferments, it is always associated with more or less of the glucoses.

Preparation.—The cane sugar of commerce is, at present, almost entirely produced from either the sugar cane or the beet, small quantities only of sorghum, palm, or maple sugar ever coming into market. The sugar cane has the great advantage over the beet in that its juice is both richer (14 to 20% of sugar) and purer. When beets were first introduced for this purpose, they contained only from 5 to 6% of sugar, and, though now they have been improved till they contain 15 to 16%, still the juice is very impure and needs a very careful treatment.

The process, in brief, consists of extracting the juice by crushing or diffusion, of purifying it by coagulating with lime and heat, skimming, and filtering, and finally of evaporating it down nearly to dryness at as low a temperature as possible. The crude sugar must afterwards be refined by dissolving in water, filtering off the impurities very carefully with cloth and bone-black filters, and then evaporating in vacuum pans.

Properties.—Cane sugar, from whatever source it is derived, is a white crystalline substance, of a specific gravity of 1.606. It is sweet, extremely soluble in water, but practically insoluble in strong alcohol and in ether. It crystallizes readily from its solutions when pure, but this crystallizing is easily hindered by numerous substances, both organic and inorganic, especially by solutions of lævulose or of invert sugar. It polarizes to the right, $[\alpha]_D$ being equal to $+66.5°$. It is readily decomposed, into equal quantities of dextrose and lævulose, by long-continued boiling of an aqueous solution, by dilute acids, especially if heated, and by a variety of organized and unorganized ferments. The resulting product polarizes decidedly to the left, the influence of the lævulose being greater than that of dextrose, so that the plane of polarization is, by this operation, changed from right to left, or inverted, and hence the product is called invert sugar.

When cane sugar is gently heated, it melts at about 160° C. to a clear, almost colorless liquid, which solidifies on cooling to a clear glass, liable, however, to become cloudy and crystalline on gently warming again. This product is commonly known as

barley sugar. On heating a little further, the color changes to yellow, and the sugar is converted into dextrose and a substance known as lævulosan, a gummy material, with a formula of $C_6H_{10}O_5$, which polarizes slightly to the right, and changes readily into lævulose on boiling with water, or when treated with weak acids, or by the prolonged action of yeast. When the mass is further heated it darkens in color, giving off gases, and changing to caramel, a name given to a group of strongly colored substances, soluble in water, which have the formula of sugar, less some molecules of water, *e.g.*, $C_{12}H_{18}O_9$, $C_{12}H_{16}O_8$, and so on. Finally, if the heating is continued far enough, all the hydrogen and oxygen are driven off, carrying with them part of the carbon, and leaving behind a fine, slowly combustible coke equal in weight to about one-third of the original sugar.

Tests.—These caramel products can also be easily formed from cane sugar by the action of concentrated sulphuric acid, which has at all times a great affinity for water. Dextrose is not so readily affected by this acid; so this test serves to distinguish between the two substances. On the other hand dextrose is readily changed to caramel compounds by the action of caustic potash, as illustrated before in Moore's test. Pure cane sugar does not respond to the various glucose tests, nor will it undergo alcoholic fermentation until it is inverted by the invertin ferment of the yeast plant.

Uses.—Cane sugar is used almost entirely for food—not only for its sweetening qualities, but also on account of its slight antiseptic properties, in preserves and syrups. It is somewhat used to strengthen as well as to sweeten wines, and in an impure form it often serves as a source of alcohol. Some of it is used to produce caramel, which is valuable for its powerful coloring properties, as well as, to a slight extent, for its flavor; and it has been employed in the manufacture of explosives.

MILK SUGAR OR LACTOSE.—$C_{12}H_{22}O_{11} + H_2O$.

Occurrence and Preparation.—This sugar occurs in the milk of mammalia, and has also been found in small quantities in the fruit of one or two rare plants. The percentage in milk varies

from 3–5% in the case of sheep and cow's milk, up to 6 or 7% or even higher in the case of human and mare's milk.

It is usually obtained as a by-product, in the manufacture of cheese, by evaporating the whey and letting the sugar crystallize out on sticks or strings. This crude sugar can then be purified by recrystallizing.

Properties.—Milk sugar occurs in more than one modification, two forms of the anhydride being known, besides the ordinary form, which contains one molecule of water. The latter consists of large rhombic crystals, of a specific gravity of 1.53, and of a slightly sweetish taste. It is not very soluble in water, and hence gives a peculiar, rather gritty feeling between the teeth. It polarizes to the right about the same as anhydrous dextrose, $[\alpha]_D$ at 20° C. being equal to + 52.5°. This rotation is increased on inversion, the lactose being converted into equal quantities of dextrose and galactose.

On heating dry lactose to 130° C., the molecule of water is driven off and we have one of the modifications of the anhydride. On further heating, it changes color and is gradually converted into lacto-caramel, a substance very similar to the caramel from cane sugar. Finally, a residue of carbon only is left.

Tests.—Milk sugar responds readily to all the different glucose tests described in Lesson II. (The phenyl lactosazon which results from long-continued heating with phenyl hydrazin and acetate of soda can be distinguished from the glucose compound as it forms, on cooling, yellow, spherical masses of needle-shaped crystals.) It differs from glucose by not directly undergoing alcoholic fermentation with yeast, and by fermenting to lactic acid when its solutions are exposed to the air. It is claimed by some authors that certain varieties of yeast will produce alcohol from it, but probably in all cases it must first be inverted to glucose before the yeast will attack it.

Uses.—It is a valuable article of food when in the form of milk. In a dry state it is used to some extent in pharmacy as a medium for taking drugs. It is sometimes used, in koumyss and similar products, as a source of alcohol.

MALT SUGAR OR MALTOSE.—$C_{12}H_{22}O_{11} + H_2O$.

Occurrence and Preparation.—This sugar, although belonging to the di-saccharid group, is so very similar to dextrose that for a long while no distinction was made between the two.

It is produced from starch by the action of the various amylolytic ferments already described, diastase, ptyalin, and amylopsin, and also from glycogen by the same ferments and probably in the liver. In all these cases, however, the maltose is changed into dextrose by prolonged action. It is also produced as an intermediate product in the conversion of starch into dextrose by means of acid, and hence occurs in small quantities in the raw glucose and glucose syrups.

Properties.—Maltose forms white, needle shaped crystals, readily soluble in both water and alcohol. It polarizes strongly to the right, $[\alpha]_D$ at 20° C. being equal to + 138°. It responds to all the ordinary glucose reactions, even, after long-continued warming, to the phenyl hydrazin test, where the phenyl maltosazon finally separates out on cooling into yellow, needle-shaped crystals, which do not radiate, like those from glucose, but remain separate.

By the action of yeast, maltose ferments easily and completely, and hence, besides its value in the digestion of carbohydrates, it is chiefly important as a means for obtaining alcohol from starch.

Starch as such is quite incapable of undergoing alcoholic fermentation until it is changed to maltose by the action of the diastase ferment of malt, or, in a few rare instances, by the ptyalin of the saliva.

FERMENTATION EXPERIMENT.

In this experiment the materials, proportions, and temperatures used are the same as those employed in the manufacture of corn whiskey. Indian corn is taken as the cheapest and most convenient source of starch, but with every eight parts of corn is mixed one part of rye meal, not for the purposes of flavor, but because the salts and proteids contained in the rye enable the yeast to grow better. The mixed meal is "mashed," *i.e.*, stirred to a stiff, smooth paste with boiling water, in order to hydrate all the starch present and let the diastase act promptly. It is

then cooled to 65° C., and the crushed malt, equal in amount to the rye meal, is mixed in. The diastase attacks the starch almost at once, thinning down the mash by converting the paste into a solution of maltose. It is then further cooled to 24°, when the yeast is added, and the fermentation begins almost immediately, and can be traced by the evolution of carbon dioxide. The fermentation is completed in from two to three days, by which time a "beer" has been formed containing 4 or 5% of alcohol. This is then concentrated to the desired degree by distillation.

LABORATORY EXPERIMENTS.

CANE SUGAR, MILK SUGAR, AND FERMENTATION.

I. **Cane Sugar or Sucrose.**—1st. *Tests on dry sugar.*

(*a*) Place a little sugar in the smallest evaporating dish and heat very gently. Notice that the sugar gradually melts into a clear, yellowish liquid (when cold, called barley sugar), and afterward changes into glucose and into caramel. Let it cool, add a little water to it, and test the liquid for the glucoses (invert sugar) by the qualitative glucose tests.

(*b*) Put a little sugar into one test-tube and some dry glucose into another. To each add a little common H_2SO_4. Notice that the sugar blackens at once, while the dextrose is not affected for some little time.

(*c*) Put a little sugar into one test-tube and some dry glucose into another. To each add a little KOH; heat, and notice that only the dextrose changes color.

2d. *Dissolve some sugar in water, and test the solution.*

(*a*) Try it with Fehling's solution; notice that it does not respond.

(*b*) Boil the sugar solution first and then test; it responds either not at all or only slightly.

(*c*) Add to the solution a drop of H_2SO_4 dil. or of HCl dil., and just raise it to the boiling-point. Now test with Fehling's solution and there will be a strong reaction for glucose (invert sugar).

II. **Milk-Sugar or Lactose.**—Taste it; dissolve some in water; test this solution with Moore's and Fehling's tests.

III. **Fermentation Experiment.**—Fill the saucepan one-half full of water, boil it well, remove the flame, and at once stir into it all the corn-meal and the rye flour. Stir it thoroughly, boiling all the time, till all the lumps are broken up and the "mash" is smooth and stiff. Cool to 65° C., and then add the malt, ground up, and with the husks sifted out. Notice how the malt at once "cuts" the mash, changing the starch into maltose. Stir and cool to 24° C., preparing the bent delivery-tube while it cools. Pour it into the bottle, add the yeast, thinned with a little water, and finally fit in the cork and delivery-tube. The end of the latter must dip



FIG. 3.

into lime-water, $Ca(OH)_2$, placed in a conical glass. Cover this with a piece of filter-paper, with a hole through which the delivery-tube can pass; and at intervals during the rest of the afternoon, and during the next day, notice the progress of the fermentation.

The delivery-tube mentioned above is a quarter-inch tube of soft glass some fifteen to eighteen inches long, which the student is expected to bend into shape in the Bunsen flame, or, better, in a flat, open gas jet. It should be bent first at a right angle, some three inches from the end that is to fit into the cork, and some four or five inches farther on a second bend should be made, through an angle of 45° or so, to lead the gas down into the lime-water.

LESSON V.

ALCOHOL, CARBON DIOXIDE, AND YEAST.

ALCOHOL; ETHYL ALCOHOL.—C_2H_5OH.

History.—The name alcohol in chemistry has been given to a large and important series of organic compounds formed by the combination of hydroxyl, OH, with basic organic radicals, and corresponding exactly with the inorganic compounds known as the metallic hydrates or hydrolides.

The hydrate of ethyl, C_2H_5, or ordinary ethyl alcohol, which is the only member of the series that interests us here, has been known in a dilute and impure state, as wine and beer, since the earliest ages. It was not possible to obtain it in at all a pure form until after the invention of distilling by the early alchemists. The "spirits of wine," as it was then called, was early recognized as a powerful medicine, and it was as a medicine that the use of distilled liquors first spread over the civilized world. By repeatedly rectifying over wood ashes or lime, the early alchemists were able to obtain even absolute alcohol in a fairly pure condition.

Preparation. — Alcohol can be made from purely inorganic substances, as, *e.g.*, from acetylene gas originally derived from the combination of carbon and hydrogen. In practice, however, it is exclusively prepared by the alcoholic fermentation of certain carbohydrates, through the agency of the common yeast plant, Saccharomyces cerevisiæ.

This plant, first described by Van Leeuwenhoek, consists of small oval cells, varying in length from .008 to .014 mm. It is usually found in bunches or aggregations known as top yeast, because the bubbles of gas catching in them raise them to the surface. Sometimes, however, when the fermentation takes place at a very low temperature, the yeast cells break off from each other as fast as they form, and the individual cells settle downward, making what is known as bottom yeast. Under ordinary

circumstances yeast grows best at a temperature of from 23° to 24° C., though it still lives and multiplies at temperatures considerably above and below this.

It feeds mainly on certain carbohydrates, namely maltose and the glucoses, which it converts into alcohol and carbon dioxide, according to the formulæ:

For maltose, $C_{12}H_{22}O_{11} + H_2O = 4\ C_2H_5OH + 4CO_2$

For glucose, $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$.

Besides carbohydrates, the yeast needs small quantities of fats, of nitrogenous material, and of mineral salts. Also, besides alcohol and carbon dioxide and its own substance, it produces small quantities of the higher alcohols, of organic acids, of glycerin, and of other organic compounds.

The quantity of alcohol in a liquid that has undergone fermentation rarely, under the most favorable circumstances, amounts to 10%, and usually is not much more than half that amount. By simple distillation, it is possible to concentrate the alcohol to any desired amount up to 93% or 94%. Above this point it is necessary to add to the still some hygroscopic substance, like quicklime or chloride of calcium, to absorb the rest of the water.

Properties.—When pure it is a colorless volatile liquid, of a pleasant smell and very burning taste. It has a specific gravity at 15° C. of .793, and, as this increases regularly with the quantity of water mixed with it, we can readily, if we know the density, determine the percentage of alcohol. It boils at 78.3° C., it burns readily with a blue flame, and its vapors form explosive mixtures with air. Its freezing point has not been determined. It has a strong affinity for water, absorbing it from the air, or from substances it may be in contact with. Hence alcohol is largely used as a dehydrating, hardening, and preserving medium, and hence also comes the burning sensation when strong alcohol is brought in contact with the mucous membranes of the body. It is a curious fact that when alcohol and water are mixed together a perceptible shrinkage occurs.

Uses.—Alcohol is principally used as a stimulant, being consumed in enormous quantities under the form of both distilled and fermented beverages. It is exceedingly valuable as a medicine. It is largely used in the arts as a solvent, and also in small

amounts for the preparation of chloroform, iodoform, and some other organic compounds.

Tests.—The tests for alcohol are of considerable importance.

1st. *The Iodoform Test.*—Iodoform, CHI_3, a compound of iodine and the organic radical methenyl, is formed by the action of iodine and potassic hydrate on alcohol, and also on acetone, aldehyde, and some other organic compounds. The reaction is as follows:

$$C_2H_5OH + 4I_2 + 6KOH = \underset{\text{Iodoform}}{CHI_3} + \underset{\text{Potassium formate}}{KCHO_2} + 5KI + H_2O$$

The iodoform precipitates out in the form of yellow, six-sided crystals. These crystals first appear as plain, flat hexagons, but, after standing a little, modifications occur on each of the six sides, forming regular star-shaped figures of much beauty.

Iodoform is very heavy, being almost entirely composed of iodine. It is soluble in alcohol, very slightly soluble in water, has a peculiar smell, and is very largely used in surgery as a mild antiseptic.

This test is extremely delicate, but reacts with acetone and aldehyde as well as with alcohol.

2d. *The Molybdic Acid Test.*—In this test we use a solution of molybdic anhydride, MoO_3, in sulphuric acid. The alcohol takes an atom of oxygen from the compound, changing it to deep blue suboxide of molybdenum, MoO_2, and itself being converted into aldehyde, a volatile liquid of unpleasant odor.

The reaction is as follows:

$$C_2H_5OH + MoO_3 = MoO_2 + \underset{\text{Aldehyde}}{C_2H_4O} + H_2O$$

This test is quite delicate, but reacts with other strongly reducing organic substances besides alcohol.

3d. *Chromic-Acid Test.*—This also depends upon the reduction of a chemical substance by the alcohol, which is oxydized to aldehyde.

In dichromate of potash, $K_2Cr_2O_7$, or dichromic acid, $H_2Cr_2O_7$, which may result from its mixture with hydrochloric acid, the oxide of chromium acts as an acid radical. On reduction, however, the metal itself is set free, and this combines with the hydrochloric acid to form a green chloride of chromium. Thus:

$$K_2Cr_2O_7 + 3C_2H_5OH + 8HCl = Cr_2Cl_6 + 3C_2H_4O + 7H_2O + 2KCl$$

This test is not as delicate as the preceding ones, and reacts with most of the ordinary reducing agents, hydrogen, sulphurous acid, glucose, and others.

4th. *Acetic-Ether Test.*—In this test, by the action of the acetic acid on alcohol, we produce a so-called compound ether, *i.e.*, an oxygen salt of an organic radical; in this case, an acetate of ethyl, or acetic ether. The reaction is precisely similar to the formation of potassic nitrate by the action of nitric acid on potassic hydrate.

Thus, $\underset{\text{Potassic Hydrate}}{KOH} + \underset{\text{Nitric Acid}}{HNO_3} = \underset{\text{Potassic Nitrate.}}{KNO_3} + H_2O$

$$\underset{\text{Ethyl Alcohol}}{C_2H_5OH} + \underset{\text{Acetic Acid}}{H.C_2H_3O_2} = \underset{\text{Ethyl Acetate or Acetic Ether}}{C_2H_5.C_2H_3O_2} + H_2O$$

The strong sulphuric acid is added partly to set free acetic acid from the acetate of soda, but chiefly to assist the reaction by absorbing the water liberated.

Acetic ether has a particularly pleasant, fragrant smell, not unlike that of bananas; it occurs in minute quantities in the flavoring elements of certain fruits, and also in the bouquet of old wines and liquors.

LABORATORY EXPERIMENTS.

ALCOHOL, CARBON DIOXIDE, AND YEAST.

I. **Alcohol.**—C_2H_5OH.—Decant off the top of the fermented liquor, from the last lesson, into a flask. Add a piece or two of pumicestone, fit in the distilling-tube, and distil gently half the liquid into a flask, small beaker, or test-tube. Taste the distillate and test it for alcohol as follows:

1st. *Iodoform Test.*—Add to a test-tube one inch of the distillate and five or six drops of KOH. Warm very gently, add a little iodine till it is yellow, and then, carefully, one or two drops of KOH till the yellow color just fades. Let it stand a few minutes, mix it up gently, notice the characteristic odor of iodoform, and examine the crystals under the microscope.

2d. *Molybdic Acid Test.*—Put in a test-tube half an inch of molybdic acid solution. Add two or three drops of the distillate; notice the blue color, due to MoO_2.

3d. *Chromic-Acid Test.*—Fill a test-tube one third to one-half full of the distillate, add two or three drops of potassic dichromate, $K_2Cr_2O_7$, and two or three drops of HCl conc. Boil; notice that the liquid turns green.—N.B. This test is not as delicate as tests 1st and 2d. If it does not work well with the distillate, repeat it with a little alcohol from the shelf.

Make the following test with alcohol from the shelf:*

Acetic Ether Test.—Fill a flask one-third full of alcohol, add one-fourth as much sodic acetate, $NaC_2H_3O_2$, and then run in, very carefully, common H_2SO_4, shaking gently till the liquid begins to boil. Notice the pleasant fragrant odor of acetic ether.

II. **Carbon Dioxide.**—CO_2.—Take the conical glass of $Ca(OH)_2$, exposed to the gas from the fermentation, and filter off the white calcic carbonate, $CaCO_3$, in a small funnel. Throw away the filtrate, but set the funnel over a small test-tube, and on to the filter paper pour a few drops of HCl dilute. Notice the effervescence that results, showing the presence of CO_2. Boil for a minute the solution of calcic chloride that passes through into the test-tube, to drive off the CO_2, add to it some ammonic hydrate, NH_4OH, till it is alkaline to red litmus paper, and then a few drops of ammonic oxalate, $(NH_4)_2C_2O_4$. The white ppt. of calcic oxalate that results shows calcium, Ca.

III. **Yeast.**—With the platinum wire pick out a little yeast from the top of the liquid in the fermentation bottle, or from the sides of the bottle, where the top of the liquid has been. Spread this out on a slide with a drop of water, and examine it under the microscope with both low and high powers, both without and with the addition of a little fuchsin. Notice the yeast cells, both single and in chains and masses. Also take a drop of the liquid from the fermentation bottle, and, examining carefully with the high power, notice the great number and the large variety of bacteria present.

* When heating solutions containing much alcohol, or when using volatile liquids, especially gasolene and ether, great care must be taken to prevent the vapors from igniting.

N.B.—The distilling-tube mentioned in the first part of the above lesson is to be prepared by the student from a piece of quarter-inch soft glass tubing some thirty inches long. When properly made and fitted to the flask it should have four bends in it, as follows: About an inch from the cork it should bend upward on a gradual incline, so as to give vapors that condense readily a chance run to back into the flask. Then, near the

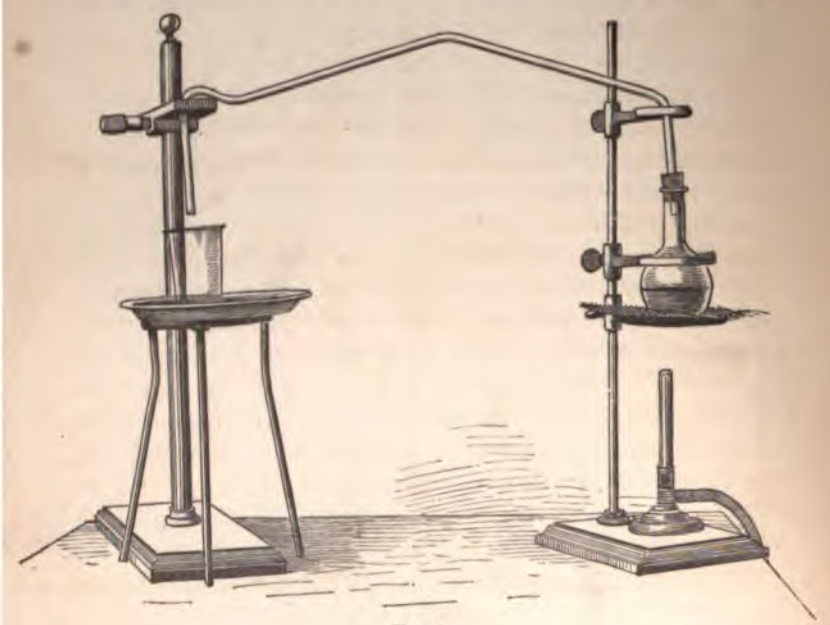


FIG. 4.

middle of the tube, it should be bent downward at a similar angle. On this part are hung, during the distillation, pieces of filter paper kept wet by a constant stream of water from the wash bottles. About four or five inches from the end of the glass it should be bent upward for half an inch or so, so as to give a drip for the wash water; and then the tube is bent down perpendicularly to let the distillate drop down into a beaker, flask, or test-tube below.



FIG. 1. Phenyl Glucosazon, x 50.
(Large crystals.)



FIG. 2. Phenyl Glucosazon, x 175.
(Small crystals.)



FIG 3. Iodoform, x 225.



FIG. 4. Mould Plants, x 100.



FIG. 5. Yeast, x 250.



FIG. 6. Bacteria, x 500.

C. E. P., del.



LESSON VI.

FATS AND SOAPS.

THE FATS AND FIXED OILS.

These substances differ from the carbohydrates and the proteids in that both their composition and their chemical structure are well understood. They belong to the compounds mentioned on the last page as compound ethers, *i.e.*, oxygen salts of an organic radical, and, though of varied composition, they have this in common: they are all salts of the one triatomic radical propenyl, C_3H_5, with the large class of acids known as the fatty acids.

Occurrence.—The fats occur in large quantities and in great variety in both animals and plants. They seem, in most cases, to be present as a store of highly-oxidizable food. In animals they occur to a small extent in the fluids of the body—blood, sweat, lymph, etc.—and also, in the form of an emulsion, in milk. They are present in almost all the tissues of the body, and are found as special deposits in large quantities in various parts of the body in the cells of the adipose tissue. They are stored up in plants chiefly round the embryo in seeds and nuts, where they take the place, more or less completely, of starch.

Preparation.—The animal fats are extracted from the tissue in several ways. The simplest method, and, until late years, the only one practised on a large scale, is to try it out by heating it until the other matter in the cells shrivels up and sets the fat free. The disadvantage in this method is that, no matter how carefully it is managed, the temperature must be raised so high that some decomposition takes place, seriously injuring the flavor if not the appearance of the fat.

Some fats, as palm oil, are extracted by boiling the material with water, when the oil rises to the surface. In other cases they are extracted by pressure.

When the fats are valuable, they are sometimes even dissolved out by some solvent, such as carbon disulphide.

A great improvement in the extraction of ordinary fats was introduced by Mège Mouriez, of Paris, about twenty years ago, and consists of the hashing the adipose tissue extremely fine and then heating it gently to a temperature just above the melting-point of the fats. In this way the cells are broken up mechanically, and the melted fat runs out without any trouble, giving a particularly pure article. This process is the basis of the oleomargarin industry, and has been applied to the extraction of fat from other sources.

Composition.—The chemical nature of these bodies was first determined by Chevreuil, about 1820, who found that most of the common fats and oils, no matter what their consistence, were composed of olein (liquid) associated with stearin, margarin, and palmitin. Heintz afterwards eliminated margarin, showing it to be a mixture of stearin and palmitin.

The ordinary animal fats contain these three substances and no others. In butter we find mixed with them small proportions of other fats, which give it peculiar properties, and in many vegetable oils, linseed and castor oils, for instance, we find quite large quantities of other similar compounds mixed with them. But in most cases these three substances so far predominate that the physical properties of the fat depend upon the varying proportions of the hard stearin, the fluid olein, or the soft, semi-solid palmitin present.

These fats are, as already mentioned, salts of the base propenyl, C_3H_5, with three molecules each of stearic, oleic, and palmitic acids respectively. Thus the formula for stearin is $C_3H_5.(C_{18}H_{35}O_2)_3$, derived from C_3H_8 and three molecules of stearic acid, $H.C_{18}H_{35}O_2$. In percentage composition the carbon predominates, with hydrogen and oxygen in smaller proportions; stearin, for instance, contains carbon 78%, hydrogen 12%, and oxygen 10%. As seen by the formula there is less oxygen, and hence greater potential energy, in these than in the carbohydrates.

Properties.—The fats all weigh less than water, their specific gravity varying from .910 to .970 or so. When pure they are colorless or faint yellow, and generally have but little taste or smell.

They are viscous and unctuous to the touch, leaving a grease spot on paper, and not evaporating below a decomposing temperature. With the exception, perhaps, of castor oil, they have no action on polarized light.

They are quite insoluble in water, but dissolve more or less readily in boiling alcohol. They dissolve freely in many volatile organic liquids, like the light petroleum and coal-tar products, ether, carbon disulphide, chloroform, and others. In bulk they are not, as a rule, specially inflammable, but burn freely in small quantities, *e.g.*, with a wick. They readily decompose, when heated dry, into a variety of more or less offensively smelling bodies; this also occurs when they are treated with strong sulphuric acid or other dehydrating agents. When acted on by superheated steam or by alkalies and dilute acids they undergo saponification, *i.e.*, they change to glycerin and either fatty acid or soap. On long-continued exposure to the air, some of them, the so-called drying oils, are gradually oxidized into more solid compounds; while others, unless exceedingly pure, undergo a process of fermentation and are more or less decomposed into fatty acids and other compounds.

Uses.—The fats are among the important constituents of animal food, although a good deal of the fat in the body is undoubtedly formed in the system from carbohydrates and proteids. They serve as fuel, being oxidized to carbon dioxide and water, and, owing to their small percentage of oxygen, they develop a large amount of energy. Often the fat must be added to the food, before eating, in the form of butter, suet, and oils; this is generally the case with vegetable food. Animal foods are, as a rule, richer in fat, ordinary meat containing from 5 to 10%, milk 3 to 4%, eggs 12%, cheese 8 to 30%, and butter 85 to 90%.

Besides as food, some of the fats are used in medicine for certain special properties they possess, *e.g.*, castor oil and cod-liver oil. In pharmacy also they are largely employed as ointments and salves, and as a medium for other and more active remedies.

Enormous quantities of fat are consumed in the manufacture of soap; the oils are extremely useful for lubricating purposes, and as vehicles for paint, and, though now largely superseded by gas and petroleum products, for artificial illumination.

The waxes are similar to the fats in being compound ethers of the fatty acids. These acids, however, are not combined with propenyl, but with cetyl, $C_{16}H_{33}$, ceryl, $C_{27}H_{55}$, myricyl, $C_{30}H_{61}$, and one or two similar radicals.

SOAP.

Definition.—A soap may be defined as a metallic salt of a fatty acid. This distinguishes it from a fat, which, as we have seen, is a salt of a fatty acid with propenyl, and from glycerin which is the hydrate of propenyl. Although the number of possible soaps is very large, the only ones commonly known and in general use are those of the alkaline metals, sodium and potassium.

Preparation.—Soap is generally made by boiling fat with a caustic alkali, either with potash, when the soft or potash soap is produced, from which the hard soap must be prepared by the aid of salt, or, as is now more generally the case, with soda. Ordinarily this process takes a good deal of time, and, to hasten the operation, when but small quantities are to be made, the alkali is sometimes dissolved in alcohol—as, for instance, in Lesson VII. It is possible, however, when using just the right proportions of castor oil and very concentrated sodic hydrate, to make a hard castor-oil soap, ricinoleate of soda, in a very few minutes, without much heating.

When a fat is saponified in this way, a very simple reaction takes place, the metal of the alkali changing places with the propenyl of the fat. As propenyl has three bonds, however, and potassium or sodium has only one, we must have three potassium atoms present for every molecule of the fat.

$$\underset{\text{Propenyl Stearate or Stearin}}{C_3H_5(C_{18}H_{35}O_2)_3} + \underset{\text{Potassic Hydrate}}{3KOH} = \underset{\text{Potassic Stearate or Soap}}{3KC_{18}H_{35}O_2} + \underset{\text{Propenyl Hydrate or Glycerin}}{C_3H_5(OH)_3}$$

These potash soaps are soft and semi-fluid at ordinary temperatures, and hence are called soft soaps. If sodic hydrate were used instead of the potash, we should form a soda or hard soap, like the ordinary toilet or cake soap. This can also be prepared from the soft soap by the addition of common salt, NaCl.

Thus, $\underset{\text{Soft Soap.}}{KC_{18}H_{35}O_2} + NaCl = \underset{\text{Hard Soap}}{NaC_{18}H_{35}O_2} + KCl$

Properties.—Both of these varieties of soap dissolve in warm and cold water, and in alcohol. When dissolved in large quantities of water, they have rather a turbid appearance and an alkaline reaction, and seem to be converted, more or less, into free alkali and an insoluble acid salt. This property, it is believed, enables them to act as cleansing agents; for the free alkali attacks the greasy dirt on hands or clothing, dissolving the fatty matter and setting the rest free, while the insoluble salt forms a slippery lather, which involves and carries away with itself the dirt and gritty matter. There is always present, also, in commercial soaps, a certain quantity of free alkali, which contributes to the cleansing effect. They always contain water, from 20 to over 80%, as well as salts, coloring and scenting materials, and often solid matters—sand, emery, etc.—either for special purposes or for adulteration. On the addition of acid, they decompose, yielding free fatty acids, according to the following reaction:

$$\underset{\text{Soft Soap}}{KC_{18}H_{35}O_2} + HCl = \underset{\text{Stearic Acid.}}{HC_{18}H_{35}O_2} + KCl$$

These ordinary soda and potash soaps are the ones so largely used for washing and cleansing purposes. By adding metallic salts to their solutions we can form insoluble soaps with almost every metal in the list of elements. Several of these compounds were prepared in the lesson, and in each case the metal is simply substituted for the potassium or sodium of the soap, as in the following reactions:

For silver,

$$AgNO_3 + KC_{18}H_{35}O_2 = AgC_{18}H_{35}O_2 + KNO_3$$

and for calcium,

$$CaCl_2 + 2KC_{18}H_{35}O_2 = Ca(C_{18}H_{35}O_2)_2 + 2KCl$$

Two of these soaps deserve a little notice: The lead soap is prepared sometimes in pharmacy for use as a plaster, not, however, as in this lesson, but by boiling together oxide of lead with water and oil. The calcium or lime soap, generally mixed with the magnesium soap, is the compound formed when soap is used in hard or calcareous water. The lime in the water at once forms this sticky, insoluble precipitate, nor is it possible to get a satisfactory lather until the lime has taken up its full share

of soap. By adding, however, a certain amount of carbonate of soda to the water, the lime will be precipitated or neutralized and the lather forms at once.

EMULSION.—Before finishing the lesson, a word or two of explanation should be given about this peculiar condition. A fluid or semi-fluid substance is said to be emulsified when its particles are finely divided and refuse to coalesce. When this happens, it is supposed that each little particle is coated with a thin layer of some other body, which is sufficient to prevent a true contact between the globules. This is done, for instance, when mercury is rubbed up in a mortar with lard, forming a gray ointment, with minute globules of mercury kept apart by a coating of fat. In oils and melted fats this occurs best when they are shaken up with solutions of soap, or, which amounts to the same thing, of alkalies or alkaline salts, and also of various organic compounds, such as proteids, gums, etc. Sometimes as in milk, both alkalies and proteids unite to make a very perfect emulsion. On examining a drop under the microscope, the little globules of fat can be seen, perfectly distinct, of various shapes and sizes, and quite separated from each other.

LABORATORY EXPERIMENTS.

FATS AND SOAPS.

I. **Beef Fat.**—Cut up the beef fat into small pieces, place it in the saucepan, and heat it carefully over the flame until the envelopes shrivel up and the fat melts out. Do not heat too hot, and be very careful not to let any water get into the saucepan during the operation. When all the tallow is free, filter it through dry filter paper on to the agate plate, and let it cool for an hour. Then put a little shaving of this fat into the "melting-point tube," tie the tube to the thermometer so that the lump of fat is level with the bulb of the instrument, and place both together in a beaker of water, not letting the water enter the tube. Heat very gently, and notice the temperature at which the tallow begins to melt, usually between 40° and 45° C.

II. **Soaps.**—Dissolve soft or potash soap in warm water in a beaker, and test the solution as follows:

1st. Put in a test-tube half an inch of solution, add water till half full, and shake. Notice the strong lather.

2d. Put in a test-tube half an inch of solution, add a little calcic chloride, $CaCl_2$, and then water. Shake = no lather, but a white curdy ppt. of lime soap.

3d. Put in a test-tube half an inch of solution and a little $CaCl_2$. Add half an inch of sodic carbonate, Na_2CO_3 (washing soda), and water, and shake = a lather, as in 1st. The soda has neutralized the effects of the lime.

4th. Fill a test-tube half full of solution, and add a few drops of HCl conc. Notice the separation of fatty acids. Heat gently; notice that the acids will float like oil on the top of the liquid.

5th. Make a series of metallic compounds (soaps) by putting some soap solution into 8 test-tubes and adding separately a few drops of the following reagents:

(*a*) Baric chloride, $BaCl_2$ = a white Ba soap.

(*b*) Magnesic sulphate, $MgSO_4$ = a white Mg soap.

(*c*) Ferrous sulphate, in solution, $FeSO_4$ = a greenish-white ferrous soap.

(*d*) Ferric chloride, Fe_2Cl_6 = a brown ferric soap.

(*e*) Plumbic acetate, $Pb(C_2H_3O_2)_2$ = a white Pb soap (lead plaster).

(*f*) Cupric sulphate, $CuSO_4$ = a blue Cu soap.

(*g*) Argentic nitrate, $AgNO_3$ = a white Ag soap.

(*h*) Mercuric chloride, $HgCl_2$ = a white mercuric soap.

N.B.—If the soft soap used in these tests contains much free caustic alkali, the argentic and mercuric ppts. will be colored gray and red respectively from the presence of AgOH and $Hg(OH)_2$.

To the rest of the solution in the beaker add a quantity of dry sodic chloride, NaCl. Boil and notice the formation of hard, or soda, soap.

III. **Formation of Hard Soap.**—Make some soap in the following manner:

To the castor oil, in a small evaporating dish, add the strong solution of sodic hydrate, NaOH, and stir thoroughly with a glass rod. Notice that at first this forms simply an emulsion,

but that gradually this emulsion gets thicker and thicker. Warm it very gently on a water-bath (not letting it melt), stirring it constantly all the time, and in a few minutes the mixture will get quite stiff and firm. Wait and stir a little longer and it will form a solid, hard soap. Dissolve this soap in a beaker with hot water, and with the solution make the tests 1st to 4th under Section II. and also some or all of the tests under test 5th. Notice that this soap reacts exactly the same as the soft soap.

IV. **Pork Fat.**—Scrape the saucepan clean, fill it two-thirds full of water, and boil. Tie up the pork fat in muslin, place it in the boiling water, and press it well with a rod. The lard soaks through and rises to the top. Filter through a wet filter paper, and notice that the lard does not pass through. Let some of the lard cool and notice how much purer it is than the tallow obtained from I.

Shake some of this lard, while melted, with warm water in a test-tube; notice how it rises to the top again. To the same mixture add half an inch of KOH; notice that the lard forms a white emulsion.

NOTE.—The "melting-point tube" used in the first part of this lesson is a piece of quarter-inch glass tubing some three or four inches long, drawn out into a blunt point and closed at one end. The other end is left open, and the little sliver of hard fat is dropped or pushed down it till near the bottom.

LESSON VII.

BUTTER, OLEOMARGARIN, GLYCERIN, AND OILS.

BUTTER.

Occurrence.—This fat is found in the milk of the mammalia to the amount of from 2% to 5%. It occurs, not dissolved in the liquid, but suspended in it in the form of fine round globules, kept in an emulsified condition by, probably, the casein present.

Preparation.—It is extracted first as cream, and then, in the process of churning, the globules coalesce and the solid fat separates more or less thoroughly from the other constituents of the milk.

As it comes to market, butter rarely contains more than 80% or 85% of fat; the rest is principally water, with some proteids and lactose from buttermilk still remaining in it, and with more or less salt and sometimes saltpetre, to flavor and preserve it. It is also generally colored more or less with some harmless vegetable compound. The presence of buttermilk is very objectionable, for it begins to decompose almost immediately, and sets up fermentation in the butter itself.

Oleomargarin.—Of the various fats used as substitutes for butter, the most important by far is that prepared by the so-called oleomargarin process. The fat used for this purpose is generally that contained in the intestinal folds, chiefly the omentum and mesentery, in beef cattle. It is carefully stripped from the fresh carcass, washed, chilled, and hashed exceedingly fine. It is then melted at a temperature of 50° or 51° C., and the liquid fat, drawn clear from any scrap, is allowed to stand for two or three days in small vats at a temperature (about 27° C.) at which butter is just liquid. The harder fats, stearin and some palmitin, separate in the form of fine crystals. The liquid is pressed from these, and runs away as a thin yellow oil which solidifies on cooling to crude oleomargarin (butter- or oleomargarin-oil).

To turn this into a very good substitute for butter it is only necessary to churn it with some milk so that it can absorb some of the butter taste, to add the proper amount of coloring matter, and to chill it very rapidly. It then can be salted and packed like ordinary butter. This material, as it comes into market, presents two very decided advantages over butter; for it is not only much cheaper, but, owing to the absence both of buttermilk and also of the peculiar butter fats mentioned below, it has much better keeping qualities. In flavor it ranks well with second-class butter, but hardly comes up to the standard of the very finest grades.

Composition.—Butter fat is much more complex than the other animal or even vegetable fats. Besides the ordinary stearin, palmitin, and olein, which compose over 90% of its weight, there are some eight or ten well-recognized compounds to be found in it. Among these we have the fats, myristin and arachidin; the so-called butter fats, caprin, caprylin, caproin, and butyrin; the propenyl compounds of acetic and formic acid, and the peculiar substance known as cholesterin. The butter fats, which are the most important of these, are propenyl salts of fatty acids with comparatively low molecular weights, *e.g.* of butyric acid, $H.C_4H_7O_2$; normal caproic acid, $H.C_6H_{11}O_2$; normal caprylic acid, $H.C_8H_{15}O_2$; and capric acid, $H.C_{10}H_{19}O_2$. These compounds, and especially the first of them, butyrin, give the peculiar taste and odor to butter. They are quite liable to decompose, and to develop their respective acids.

The acids themselves have some characteristic properties. They have a strong rancid smell and taste, easily recognized as occurring in decomposing butter. They are light, volatile liquids, which dissolve more or less in water, and can be distilled with the steam by boiling a solution containing them. In these respects they differ very materially from the odorless, insoluble, and non-volatile, solid or nearly solid fatty acids from the ordinary fats. Hence to determine the presence and even the percentage of butter in a sample of fat it is only necessary to obtain the fatty acids and to distil them, and then to notice the presence and the quantity of the butter acids in the distillate.

In our lesson the process is conducted as it is in practice in the

analytical laboratory. To obtain fatty acids, as in Lesson VI., we must add a strong mineral acid to soap. Hence we first convert the butter into soap by boiling it with an alcoholic solution of potash, and then, after driving off the last traces of alcohol, we decompose the soap with dilute sulphuric acid, and distil off the volatile fatty acids. If these are present, the distillate will have a rancid smell and taste, and be acid to test paper. These butter acids will form salts with bases, and with alcohol and strong sulphuric acid the butyric acid acts just the same as acetic acid did in the last test in Lesson V., *i.e.*, it forms ethyl butyrate, or butyric ether. This latter, which has a pleasant, fragrant, pineapple odor, occurs naturally in many fruits, and in liquors, especially when old and well matured, and is prepared quite largely as a flavoring agent for syrups and beverages. This odor of butyric ether is obtained more or less whenever butter is saponified in the presence of alcohol, and hence its presence on boiling a sample of fat with an alcoholic solution of potash is true evidence of the presence of butter.

GLYCERIN.

Propenyl Hydrate ; Glycerol.—$C_3H_5(OH)_3$.

Preparation.—This substance, discovered in 1779, by Scheele, is formed whenever a fat is saponified, *i.e.*, decomposed either into soap or into a fatty acid. Although produced in large quantities in the manufacture of soap by boiling with alkali, it has been found troublesome to separate this glycerin from the alkali and other impurities in a merchantable form. So it is usually prepared as a by-product in the manufacture of stearic and other fatty acids, by treating fat either with diluted sulphuric acid, or, better, with superheated water or steam. The latter reaction is as follows :

$$C_3H_5(C_{18}H_{35}O_2)_3 + 3H_2O = 3H.C_{18}H_{35}O_2 + C_3H_5(OH)_3$$

After the removal of the fatty acids, the water is evaporated, and the glycerin purified by distillation and by crystallization.

Glycerin is also produced in small quantities in alcoholic fermentation, and accordingly is present in all fermented liquors.

Properties.—When pure, it is a clear, colorless, thick liquid, with no odor and a sweet taste. It dissolves readily in water and

alcohol, and absorbs moisture from the air. It solidifies at —40° C. to a gummy mass; but, even at 0° C., crystals will slowly form if the liquid is kept quiet for a long time, and will form at once on the addition of crystals previously prepared. It boils at 290° C. and evaporates at lower temperatures, though it is practically non-volatile at the temperature of the atmosphere. It has a specific gravity at 15° C. of 1.263.

In composition glycerin is a triatomic alcohol, being a hydrate of the triad organic radical, propenyl. It ignites at 150° C. and burns readily with a blue flame. It decomposes when heated with dehydrating agents, as, for instance, sulphuric acid, into acrolein and similar compounds. It does not undergo the ordinary alcoholic fermentation with the yeast plant, and is often added as a sweetening to wines and liquors when it is undesirable to have any further fermentation. It can, however, be fermented, probably by certain kinds of bacteria, and alcohol is one of the products. By the combined action of strong nitric and sulphuric acids it is converted into nitroglycerin.

Uses.—Glycerin often takes the place of sugar as a sweetening agent and preservative in syrups and beverages of all sorts. It seems to be digested readily. It is also quite largely used as a solvent especially where there is danger of decomposition. Thus, it is used in extracting ferments, in making ointments and salves, and in dissolving delicate coloring matters, and scenting and flavoring matters, from their sources. It is also largely used, on account of its physical properties, as a lubricant, for sealing gas-holders, for filling meters, for mixing with clay, paints, etc., to keep them moist, and for several similar purposes.

THE OILS.

The oils differ from the ordinary fats in that they contain more olein and less palmitin and stearin, and hence are fluid at the temperature of the atmosphere. We have selected three of the most prominent of them to serve as examples—

Castor Oil.—This is a vegetable oil, composing nearly half the weight of the seeds of Ricinus communis. This plant came originally from the East Indies, but is now largely cultivated in hot

climates for its oil, and in more moderate climates as an ornament.

The best oil is then obtained by pressing the seeds cold, and inferior grades are obtained by heating and re-pressing the residues. It is refined by filtering, bone-black being often used.

The pure oil is a transparent, colorless or faintly greenish, thick liquid, with a slight smell and rather a nauseous taste. It has a high specific gravity, .950 to .970 at 15° C., and is extremely viscous. It solidifies at −18° C. It can easily be distinguished from other oils by being readily soluble in alcohol, and by not dissolving in the light petroleum products. Thus, when mixed with gasolene, it seems to dissolve about its own bulk of that liquid, but the mixture is insoluble in an excess of solvent.

Besides palmitin, with some stearin and olein, it contains a considerable amount of the fat known as ricinolein, derived from ricinoleic acid, $H.C_{18}H_{33}O_3$. This fat is readily saponified, even in the cold, by strong caustic alkalies, and the hard soap formed in the third part of Lesson VI. was principally composed of ricinoleate of soda.

Castor oil has important medical properties, and in its purest form it is widely used as a purgative. It is largely employed for lubricating purposes, and for soapmaking, and also in dyeing and calico printing. It is adulterated with poppy, lard, cocoanut and other oils, and also with a thicker compound, blow oil, made by partially oxidizing rape and cotton-seed oils. It gives a characteristic color with sulphuric acid.

Olive Oil.— This is extracted from the fruit of the olive-tree, either by pressing or by extraction with some solvent like carbon disulphide. It varies enormously in quality, according to the variety, ripeness, and condition of the fruit, the temperature and severity of the pressing, and the subsequent treatment of the oil. The finest grades come from the shores of the Mediterranean.

Pure olive oil has a faint yellow color, slightly tinged with green. It is almost odorless, and has a pleasant taste. It is neither as dense (sp. gr., .914 to .918) nor as viscous as castor oil. It dissolves readily in light petroleum and coal-tar compounds, as well as chloroform, carbon disulphide, and ether, but is only sparingly soluble in alcohol. It consists almost entirely of olein,

mixed with stearin and palmitin, with traces of arachidin, cholesterin, free oleic acid, and some albuminous matter. Owing to the presence of the latter, it gradually becomes rancid in the air, though it does not thicken or oxidize like the so-called "drying" oils.

Olive oil is used largely as a food, and the finer grades, used for salad oil, etc., are prepared with the greatest care, and are obtained pure with much difficulty. Inferior qualities are used for making soap, for illumination, and other minor purposes.

The price of good olive oil is so high that it is enormously adulterated, principally with cotton-seed oil, but also with lard, poppy, rape, and even purified fish or hydrocarbon oils. With a drop of sulphuric acid it turns brown at the centre, but around the drop of acid can generally be distinguished a peculiar and characteristic shade of olive-green.

Cod-Liver Oil.—This is obtained from the livers of codfish. The oil is extracted by gentle heat, and by pressure, and the resulting oil is of various grades, distinguished by color and by smell or taste.

The pure oil is of a light yellow color, with slight odor and taste, and has a faintly acid reaction. It dissolves in thirty or forty parts of alcohol, and more readily in the petroleum oils. It has a specific gravity of about .922.

It consists principally of olein, mixed with stearin, palmitin, myristin, cholesterin, and perhaps traces of the butter fats. It seems to contain traces of iodine and bromine, and also certain biliary compounds. It is used largely in medicine, principally in wasting diseases, on account of its easy digestibility. The inferior grades are used for soapmaking and for mixing with other oils.

It is adulterated with fish oils such as seal or menhaden oil, and also with oil from the livers of other fish. Lard oil and some of the seed oils are also often added to it. With sulphuric acid it gives a red spot with more or less violet around it. This is probably due to cholic acid produced from the biliary compounds present.

LABORATORY EXPERIMENTS.

TESTS ON BUTTER, OLEOMARGARIN, GLYCERIN, AND OILS.

I. **Butter.**—Put most of the butter into a flask, add about 25 c.c. (one-half the small flask full) of alcoholic potash solution, insert a cork with an upright tube in it, and boil on the water bath for twenty minutes. If the alcohol evaporates much during the boiling, add a little from the shelf. Finally pour the mixture into an evaporating dish, and dry it thoroughly, at first over the water bath, and, when nearly dry, by heating it very gently and cautiously over the sand bath, stirring constantly with a rod. Add a little of this dry residue (a butter soap) to some water in a test-tube, warm a little and shake = lather. Mix the rest with some water and return it to the flask, filling the flask about one-third full. Add about 10 c.c. of H_2SO_4 dil., and notice how the fatty acids rise to the top. Fit in the distilling-tube, as under Lesson V., and distil off the "volatile fatty acids" into a small beaker. Test this distillate as follows:

1st. Smell it; notice the rancid smell of the butyric acid.

2d. Notice its acid reaction to litmus paper.

3d. Add an equal amount of alcohol to the distillate, and then, with care, a quarter as much common H_2SO_4. While hot, notice the "pineapple" odor of butyric ether (ethyl butyrate).

Special Test for Butter and Oleomargarin.—While preparing and caring for the above, make the following tests:

Put a little butter in one test-tube, and a little oleomargarin into another. To each add one inch or so of alcoholic potash solution, and warm each in the steam of the water bath. Distinguish the one from the other by the smell. Add a little H_2SO_4 dil. to each and smell again. Notice that the "oleo" test-tube will only smell of alcohol, but that the other will smell, besides, of butyric ether.

II. **Glycerin.**—$C_3H_5(OH)_3$.—(*a*) Notice its taste, stickiness, and solubility in water.

(*b*) Dip a glass rod in it and hold it in the flame; notice that it burns with a *blue* flame.

(*c*) Heat a little in a test-tube with a few drops of common H_2SO_4; notice the acrid, irritating smell of acrolein.

III. **Castor, Olive, and Cod-Liver Oils.**—(*a*) Try to dissolve each, in separate test-tubes, with gasolene. The last two dissolve readily, but, if a good deal of solvent is used, the castor oil does not dissolve, but, combined with some of the gasolene, stays at the bottom of the test-tube.

(*b*) Try to dissolve each, in separate test-tubes, with cold alcohol. Notice that only the castor oil dissolves with readiness.

(*c*) Put two or three drops of each into separate evaporating-dishes, and add, with great care, one drop of common H_2SO_4. Notice the peculiar color produced by each oil, as follows:

Castor oil, brown in the centre, yellow outside.

Olive oil, brownish in the centre, olive-green outside.

Cod-liver oil, reddish-brown in the centre, purple outside.

(*d*) Emulsify olive oil by shaking it in separate test-tubes with water and a few drops of the following reagents:

1st. Na_2CO_3. 2d. A solution of soap. 3d. NH_4OH. 4th. Hydro-di-sodic phosphate, Na_2HPO_4.

Look at a drop of one of these emulsions under the microscope, and notice the fat globules.

PART III.

THE PROTEIDS

OR

ALBUMINOUS BODIES.

THE PROTEIDS OR ALBUMINOUS BODIES.

INTRODUCTION.

UNDER this heading we include a large and extremely important class of proximate principles, all of which have more or less resemblance to the chief constituent of the white of an egg.

Occurrence.—They are found in both the vegetable and animal kingdoms. In the former they occur in greatest abundance in the seeds, although present in smaller quantities all through the plant. In animals, however, they form a large part of the solid constituents of all the tissues and fluids, with the exception only of the sweat, urine, and bile of healthy individuals.

Although these substances occur so abundantly in animals, they cannot be formed from other classes of proximate principles, excepting by plants. Animals are obliged to absorb them already formed, and can then assimilate them and modify them as necessary.

Composition. — All proteids contain carbon, hydrogen, oxygen, and nitrogen. Sulphur is present in almost all cases, and phosphorus in a few. Besides this, they almost always leave behind, on ignition, a certain amount of ash, chiefly phosphate of lime, which in some cases may be an essential part of the compound. As, however, by great care, certain of the proteids have been obtained practically free from any mineral matter, and as the latter seems rather variable in amount, it is probably present in all cases as an impurity. Of the various elements the nitrogen is by far the most characteristic, so much so that these bodies with the albuminoids can be spoken of as the nitrogenous constituents of the body.

This nitrogen does not seem to be obtained by plants directly from the uncombined nitrogen of the air, but from the small quantities of combined nitrogen existing in both air and water in

the form of ammonia and its compounds and the salts of nitrous and nitric acids.

The average composition of the proteids is as follows (Drechsel): C, 50–55%; H, 6.8–7.3%; O, 22.8–24.1%; N, 15.4–18.2%; S, 0.4–5%.

With the exception, however, of this percentage composition, very little is known about the structure of these bodies. They must be composed of molecules of high weight and great complexity, and, from the decomposition products, we must assume that in the molecules some of the carbon atoms are arranged as in the aromatic and most of them as in the fatty compounds.

General Properties.—The proteids, with some few exceptions, are thoroughly amorphous bodies, not crystallizing, and, excepting the peptones, and possibly the albumoses and similar decomposition products, not diffusible. They all seem closely related to each other, and perhaps in the living organism can all be transformed one into the other. This, however, can only be done in a very few instances in the laboratory.

Solubility.—Some of these bodies are soluble and others are insoluble in water, and of the latter several are dissolved by dilute solutions of neutral salts, sodium and potassium chloride and sulphate, magnesium sulphate, and the like. Most of them dis solve in diluted solutions of acids and alkalies, although, in general, they are changed by this into other proteid compounds. In every case the solutions polarize to the left. Some dissolve more or less in alcohol, but they are all insoluble in ether.

Precipitation.—Several reagents can precipitate proteids from their solutions, especially on the application of heat. Among these are acids, both mineral and organic; many metallic salts, not only of the heavy metals—mercury, copper, silver, and the like—but also of the alkaline and earthy metals, especially if in excess; and many organic compounds, like chloral, phenol, picric acid, tannic acid, and in some instances alcohol. Of all these, ammonium sulphate in excess is the most efficacious, precipitating all proteids but the peptones. Also, in many cases, heat alone, in neutral or faintly acid solutions, will cause proteids to become insoluble. Sometimes when thrown down from their solutions by any of these means, the proteids are precipitated without losing their identity, but in many cases, and almost always when

heat has been applied, they are coagulated—that is chemically altered into insoluble proteid bodies known as coagulated proteids.

Decomposition.—They decompose readily into simpler products. When heated in the air they blacken, shrivel up, and emit pungent nitrogenous organic compounds, somewhat ammoniacal in structure and properties, with the well-known smell of burnt feathers. They can at last be entirely burnt away, leaving in most cases a trifling amount of ash. By boiling with dilute acids or by the action of certain unorganized ferments, such as pepsin and trypsin, they undergo a process of hydration, very similar to the conversion of starch into glucose. By this they are converted into other proteid bodies known as albumoses or the different varieties of peptones, and sometimes into the aromatic non-proteid substances—leucin and tyrosin. By organized ferments they are gradually decomposed, breaking down, by successive stages, into a large variety of final products, among which are water, carbon dioxide, ammonia, nitric and nitrous acids, sulphuretted hydrogen, and several more or less simple organic compounds.

There are certain simple reactions which, in general, can be said to be common to and characteristic of the proteids. Some of these are given in Lesson VIII., and to the most important of them the name has been given of the General Proteid Reactions.

Classification.—It is extremely difficult, with our present knowledge of the composition and properties of these bodies, to lay down any permanent scheme of classification. Indeed it seems probable that, when we learn enough about them, we shall have to classify them on the basis of their chemical structure, which, as yet, is only dimly hinted at by the nature of the decomposition products, and by the compounds they form with bases. But thus far it is only possible to divide them into a series of groups, the members of which resemble each other more or less in their solubility in different media, and their behavior toward certain reagents. Accordingly we shall first separate them into animal and vegetable proteids, and then classify them, as follows:

ANIMAL PROTEIDS.

CLASS I. **Albumins.**—Soluble in pure water; coagulated by heat.

1st. *Egg Albumin.* 2d. *Serum Albumin.*

CLASS II. **Globulins.**—Insoluble in pure water; soluble in dilute solutions of neutral salts, NaCl, KCl, Na_2SO_4, $MgSO_4$, etc. Coagulated by heat.

1st. *Vitellin.* 2d. *Crystallin* (or *globulin*). (These two are not precipitated in neutral solutions by an excess of salt, and by some authors are considered identical.) 3d. *Myosin.* 4th. *Paraglobulin.* 5th. *Fibrinogen.*

CLASS III. **Derived Albumins.**—Insoluble in water and neutral salt solutions; when freshly precipitated, soluble in dilute acids and alkalies.

1st. *Acid Albumins.* The name syntonin is generally given to the particular acid albumin formed by the action of dilute hydrochloric acid on myosin. 2d. *Alkali Albumins. Casein* is sometimes included under this group of alkali albumins, but differs from them (*a*) by coagulating at a high temperature, 130 to 150° C., and (*b*) by being coagulated by rennet in an alkaline medium.

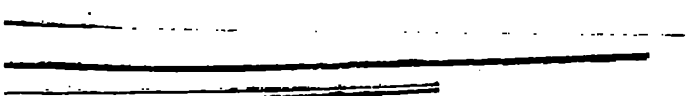
CLASS IV. **Fibrin.**—Insoluble in water; swollen by salt solutions and especially by dilute acids; coagulated on heating in water.

CLASS V. **Coagulated Proteids.**—Insoluble in water or salt solutions and hardly affected by dilute acids; dissolved with some difficulty by hot, strong acids.

CLASS VI. **Amyloid Substance** or **Lardacein.**—Insoluble in water, salt solutions, and dilute acids or alkalies; colored brownish-red by iodine.

CLASS VII. **Albumoses.**—Intermediary products between acid albumins and the peptones. All are soluble in dilute NaCl solution, and some in water. They diffuse but slightly if at all, and give a red color with the biuret test.

CLASS VIII. **Peptones.**—Soluble in water, salt solutions, acids, alkalies, and even ammonium sulphate solution. Only precipitated by tannin and by mercur-potassic-iodide. Quite diffusible, and give a red color with the biuret test.



VEGETABLE PROTEIDS.

CLASS I. **Plant Albumins.**—Soluble in water; coagulated by heat.

CLASS II. **Plant Globulins.**—Only partly soluble in water; soluble in fairly strong NaCl solutions; coagulated by heat.

CLASS III. **Plant Caseins.**—Insoluble in water and salt solutions; insoluble in dilute alcohol.

Gluten casein; legumin; congluten.

CLASS IV. **Gluten Proteids.**—Insoluble in water and absolute alcohol; soluble in dilute alcohol.

ALBUMINOIDS.

Besides the substances included in the above list, there is a large and important series of bodies, called the albuminoids, which also contain nitrogen, and which are found in both plants and animals in close and intimate relations to the proteids proper. They are distinguished from them, however, by not answering to all the general proteid reactions, and by giving different products of decomposition when treated with the digestive ferments, hot dilute acids, and similar reagents. They have not been satisfactorily isolated from each other, nor is much known about them so far. The most important of them are as follows:

Collagen, or gelatin-forming substance.

Keratin, or horny substance.

Spongin, from sponges.

Elastin, from the yellow elastic fibres of the connective tissue.

Nuclein, from the nuclei of cells, both animal and vegetable.

Mucin, from the secretion of mucous and other glands.

These occur principally in the animal kingdom, and, in general, in the intercellular substances; whereas the proteids proper occur chiefly in the fluids and in the cells. Thus the organic portion of the bone and teeth, the connective tissue, the main substance of the cartilages and tendons, the skin, hair, and nails, are all composed of these bodies. They are, with the exception of mucin, insoluble in water; and most of them, on long-continued boiling, are decomposed into substances of the nature of gelatin or glue. Some of them are not digested by the human gastric or pancreatic juices.

LESSON VIII.

THE GENERAL PROTEID REACTIONS. THE ALBUMINS AND VITELLIN.

THE GENERAL PROTEID REACTIONS.

These are certain reactions which are more or less common to all the proteids, and hence are commonly used in testing for their presence.

1st. **The Xantho-proteic Reaction.**—All proteids, whether solid or in solution, are changed, on heating with strong nitric acid, into a yellow substance called xantho-proteic acid. It is a yellow powder insoluble in water, alcohol, or ether, but easily dissolved by nitric acid, and with bases it forms a series of reddish amorphous salts, which are soluble. These salts are formed with any alkaline solution, such as the hydrates and carbonates of the alkaline metals, or the hydrates of barium and calcium. Care must be taken, however, not to boil over the liquid when adding alkalies to the hot acid solution, and also to get part at least of the mixture thoroughly alkaline before looking for the change in color from yellow to red or to deep orange.

The reaction is fairly delicate, and works with not only the proteids but also the albuminoids.

2d. **Millon's Reaction.**—This is made by heating the proteid with a little of the so-called Millon's reagent, an acid solution of nitrate and nitrite of mercury, formed by dissolving one part of mercury in two parts of strong nitric acid, sp. gr. 1.42, and diluting the solution with twice its bulk of water. The proteid is changed into a dull red precipitate, some of which sometimes dissolves in the liquid, giving that a reddish tinge. When this test is tried in the presence of much salt, it often fails, owing to the formation of mercuric chloride instead of the nitrate and nitrite.

This reaction does not answer with all albuminoid substances, but for proteids it is a characteristic test. It acts as well with

solid proteids as with the solutions, if a little water is added with the reagent before boiling.

3d. **The Biuret Reaction.**—On adding to a proteid solution a little cupric sulphate and then an excess of either caustic or carbonated alkali, the liquid is colored violet or, in the case of the albumoses and peptones, reddish. The color is intensified by boiling, but it is sufficiently delicate in most cases in the cold, and then has the great advantage of not being obscured by the presence of a reducing carbohydrate. When the proteid solution to be tested is weak, it is best to dilute the copper solution very much, and to add it in very minute quantities. If an excess of copper is admitted, the resultant blue precipitate hides the test.

In making this test on a piece of insoluble proteid, it is best to soak it for a minute or two in very dilute cupric sulphate, and then to pour that off and to add a dilute solution of sodic and potassic hydrate. The whole lump generally turns a deep violet-color.

This reaction is not quite as delicate as the previous ones. The color is the same as that obtained by the same reagents from biuret, the peculiar crystalline body obtained by heating urea.

4th. **The Ferro-cyanide Reaction.**—A few drops of potassic ferro-cyanide have the property of precipitating proteids from a solution rendered acid by acetic or hydrochloric acid. If the solution contains much sodium chloride or other salts, it is frequently necessary to dilute it with water before the proteids will precipitate. The precipitate may form quite slowly if the solution is very weak, but the reaction is still clearly perceptible.

5th. **Sodic sulphate Reaction.**—A saturated solution of sodic sulphate is also able to precipitate proteids from an acid solution. Acetic acid should be used in preference to hydrochloric, as the latter might dissolve some of the precipitate. The solution of sodic sulphate should be saturated and should be about equal in quantity to the solution of proteids. On boiling the mixture, the albuminous matter is completely precipitated and can be entirely removed by filtering. This is an important reaction in that it furnishes a way for removing proteids from a mixture without introducing any objectionable reagents.

Picric Acid Test.—This is the most important of the tests given in this lesson. Picric acid, or tri-nitro-phenol, when added in sufficient quantities to turn the solution acid, is able to completely precipitate ordinary proteids from their solutions. It is very slightly soluble, however, in water, so that, if the proteid solution is at all alkaline, it is necessary to add quite a quantity of the reagent. The presence of albumin does not interfere with subsequently testing, in the same liquid, for glucose, by adding an excess of potassic hydrate and boiling. Hence this same reagent will permit the testing for albumin and for glucose in the same test-tube—a matter of some importance when testing urines.

THE ANIMAL PROTEIDS.

CLASS I.—THE ALBUMINS.

General Properties.—These proteids occur already formed in the animal tissues and fluids. They are soluble in water and are not precipitated in the cold by very dilute acids, by sodic chloride, or by magnesic sulphate. On heating their aqueous solutions, as well as by the addition of stronger acids, they are coagulated; that is, converted into the class of coagulated proteids. If the solution is very concentrated, it is all transformed into a solid, elastic mass; if more dilute, a flocculent precipitate forms. Very much diluted solutions, or solutions from which almost all salts have been extracted by long-continued dialyzing, do not seem to coagulate on boiling. They are readily converted into the derived albumin class by heating with acids or with alkalies; they form special salts, albuminates, with many of the metals, Fe, Zu, Cu, Ag, Hg, and others; and they are readily digested by the gastric and pancreatic ferments.

EGG ALBUMIN.

Occurrence and Preparation.—This proteid occurs, in the white of birds' eggs, as a concentrated solution, enclosed in a network of delicate membranes. On cutting these membranes with broken glass or a scissors, the solution escapes and can be clarified by straining and filtering. Besides, however, the egg albumin, which is present to the extent of 12 or 13%, this liquid

contains small quantities of other proteids, probably of the globulin class, a little fatty material and soap, some mineral salts very similar to those found in the blood, and traces of glucose. To obtain pure albumin it is necessary to extract the fats with alcohol and ether, and to separate the salts by long-continued dialysis. In spite of all care, however, it is almost impossible to obtain it in a perfectly pure condition.

Properties.—When dried at a low temperature it is a light yellow, transparent, vitreous body. It dissolves readily in water to a slightly opalescent, tasteless, and odorless solution, which has usually a faintly alkaline reaction, and when concentrated is thick and somewhat viscid. Its solutions rotate to the left to an angle, so far as we can tell, of about $-36°$. On heating a concentrated solution, like the undiluted white of egg, a turbidity sets in at about 59° C., and the mass becomes solid at about 63° C. Some of the proteid present, however, is not coagulated till a temperature of 74° C. or so is reached. These temperatures, which apply only to the albumin obtained from hens' eggs, vary slightly according to the concentration of the solution and the amount of salts present. This coagulation also occurs not only with mineral acids and with alcohol, but also with ether, a test distinguishing it from serum albumin.

Composition. — The chemical composition of egg albumin (Hammarsten) is: C, 52.25; O, 23.67; N, 15.25; H, 6.9; and S, 1.93%. Very little, however, is known about its chemical structure, excepting that the molecule is large and complicated. A formula for it, given by Lieberkühn and adopted by many authors, is: $C_{72}H_{112}N_{18}SO_{22}$. This formula was obtained by assuming the sulphur to be present in the form of a single atom, and calculating from that figure for the other elements, and also by studying the different salts that it forms with some of the heavy metals. The latter, however, seem to vary in composition more or less according to the conditions under which they are formed, and it is so difficult to get both them and the albumin itself in a state of purity that the analyses cannot be considered as very accurate. As a matter of fact it is perfectly evident, from the different coagulating temperatures, that in what we know as egg albumin are contained at least two or three different proteids much resembling

each other, and these must be separated and identified before any satisfactory formula is obtained.

Among the metallic compounds mentioned, the one with mercury is of importance as the best means of counteracting the poisonous effects of corrosive sublimate, the antidote for which is uncooked white of egg.

Uses.—While egg albumin is principally used for food, it must not be forgotten that enormous quantities of it are every year employed in the arts. It is very largely consumed in dyeing and calico printing, in dressing leather, in glazing cards, bookbindings, etc., and especially in photography. In medicine it is used to some extent as an antidote, and also in dressing burns.

SERUM ALBUMIN.

Occurrence.—This proteid is found dissolved in blood serum (in human blood to the extent probably of 4 to 5%), in lymph, chyle, transudations, and, in minute quantities, in milk. It is also, under pathological conditions, found in the urine.

Preparation.—It can be prepared in a pure state from serum, by first separating the globulins with magnesic sulphate and then precipitating the albumin with sodic sulphate. It is then washed, dialyzed as thoroughly as possible, and cleansed with alcohol and ether. The serum albumin of commerce is usually only dried blood serum.

Properties.—When pure it occurs as light yellow or brownish scales, readily soluble in water to a slightly alkaline, opalescent solution, and in most respects very similar to the egg albumin before described. Its solutions rotate to the left, but the amount of rotation varies considerably according to the source of the proteid. Thus, according to the various authors, albumin from human bood rotates from $-62.6°$ to $-64.6°$; from horses' blood, $-60°$; from oxen's blood, $-57.3°$; and from dogs' blood, about $-44°$. This shows either that the serum albumin from each animal is different, or else that it is impossible to separate the true albumin from the accompanying globulins and other proteids. The temperature of coagulation also varies considerably with the strength of the solutions, the source of the albumin, and the amount of mineral salts accompanying it. Besides, however, the

difference in rotating power and in the temperature of coagulation, there are four distinct points of difference between the egg and the serum albumins.

1st. Egg albumin is rapidly coagulated by alcohol; serum albumin but slowly.

2d. Egg albumin is coagulated by ether; serum albumin is insoluble in ether, but is not actually coagulated.

3d. Egg albumin is less soluble than serum albumin in nitric and in hydrochloric acids.

4th. If egg albumin is injected into the circulation, or even if very large quantities of it are taken into the stomach, some of it may be excreted, apparently unchanged, in the urine, even in healthy individuals. This is not the case with serum albumin.

Uses.—Serum albumin, in the form of dried blood serum, is prepared in considerable quantities both for dyeing and calico printing, and for the refining of cane sugar. It enters but slightly into our supply of food.

Class II.—GLOBULINS.

These substances differ from the preceding class by being insoluble in water. They dissolve readily, however, in the presence of small amounts of neutral salts such as NaCl, Na_2SO_4, or $MgSO_4$. As it is almost impossible to separate, even by long-continued dialyzing, the last traces of these salts from proteid bodies, and as the globulins are coagulated by heat as well as the albumins, it is a matter of great difficulty to distinguish between the two classes. Hence in the case of the white of egg or blood serum, for instance, we are still in doubt as to how much of the proteid present is really albumin and how much is globulin.

All globulins are precipitated by a great excess of water, and, excepting vitellin and crystallin, by an excess of sodic chloride, or by saturation at 30° C. with magnesic sulphate. They are gradually altered by standing under water, till they lose almost entirely the property of dissolving in salt solutions. Their neutral solutions are coagulated by heat. They dissolve without change in very dilute alkalies and are reprecipitated from the solutions by dilute acids. By stronger alkalies, as well as by an

excess of mineral acids, they are changed into the corresponding derived proteids.

VITELLIN.

Occurrence.—This is found in the yolk of hens' eggs to the amount of 14 or 15%. It is associated with small quantities of the other proteids, and with the albuminoid nuclein; also with the ordinary fats, olein and palmitin, with the peculiar semi-fatty material lecithin, with cholesterin, and with some inorganic salts. Besides these, there is present a peculiar coloring matter known as lutein, which is the same as that contained in the corpora lutea, and seems to stand in close relationship to the coloring matters of blood serum, butter, and bile. It seems to owe its color to the presence of small quantities of iron; it is decolorized by sunlight and is turned blue and finally decolorized by strong nitric acid.

Preparation.—From this complex mixture we are unable to extract the vitellin absolutely pure. Hence we cannot tell positively whether it is identical with or only very closely allied to the similar proteids found in the yolk of the eggs of other birds, of fishes, of amphibians, and of reptiles. It is also very closely related to those found in chyle, and especially to crystallin, the main constituent of the crystalline lens. It is best prepared by thoroughly extracting from the yolk the fats and yellow coloring matters, and then by dissolving the cheesy white residue in 8 or 10% salt solution. To purify it, it is precipitated with an excess of water, and then redissolved and reprecipitated as often as is thought desirable.

Properties.—Thus obtained it is a white, flaky substance, a concentrated solution of which, in 10% NaCl, coagulates partially at 70° C. and wholly at 75° C. It is not precipitated by saturation with salt, and dissolves readily in dilute acids and alkalies to the corresponding derived albumins

LABORATORY EXPERIMENTS.

THE GENERAL PROTEID REACTIONS. EGG AND SERUM ALBUMIN, VITELLIN, ETC.

I. **Preparation of Egg Albumin.**—Break out, with the three-cornered file, a small hole in the shell of an egg, and through this hole pour the white, leaving the yolk inside for future experiments. Keep in a test-tube about one inch of the white for experiment (*f*). Put the rest in the bottle, shake it quietly with the broken glass, add about twice its bulk of water, and filter the mixture into a small beaker through a wet piece of muslin.

II. **General Proteid Reactions.**—Make the following tests upon the above solution, as well as on solutions of dried egg and serum albumin:

1st. *Xantho-proteic Reaction.*—Fill a test-tube half full of the solution, add half an inch of HNO_3 conc. and warm = yellow. Divide this solution among three test-tubes. To (*a*) add an excess of NH_4OH, to (*b*) add excess of Na_2CO_3, and to (*c*) add excess of KOH. Notice that in each case the yellow solution turns orange.

2d. *Biuret Reaction.*—To the solution in a test-tube add a drop of $CuSO_4$ and then half an inch of KOH = violet color. This test is more delicate if the $CuSO_4$ is diluted before using, and if very small quantities of it are used.

3d. *Millon's Reaction.*—To the solution in a test-tube add a few drops of Millon's reagent and boil = reddish curdy ppt.

4th. *Ferro-cyanide Reaction.*—To the solution in a test-tube add a few drops of acetic acid, $HC_2H_3O_2$, and then a drop or two of potassic ferro-cyanide, K_4FeCy_6 = white ppt.

5th. *Sodic-sulphate Reaction.*—To the solution in a test-tube add a little $HC_2H_3O_2$, and then an equal amount of sodic sulphate, Na_2SO_4. Boil = white ppt.

Also,

6th. Take some dry egg and serum albumin in the forceps and hold them in the flame; notice the "burnt feather" smell.

7th. Put in a small test-tube about half an inch of very strong albumin solution, with some undissolved albumin mixed with it. Add about one inch of *glacial* acetic acid and warm for a few minutes. Cool, add gently about one inch of common H_2SO_4, let it stand, and notice the fine deep violet color.

8th. *Picric-acid Test.*—To the solution in a test-tube add a few drops of picric acid = white ppt. *Compare the delicacy of this test with that of the tests Nos. 1 to 5, above.* To the solution add half an inch of KOH and boil. The ppt. redissolves and the liquid is slightly darkened in color.

III. **Special Tests on Egg and Serum Albumin.**—Make the following tests on the same three solutions:

(*a*) Add a few drops of alcohol = ppt. Notice that in the serum albumin the ppt. dissolves in an excess of water unless it has stood too long.

(*b*) Add a little $CuSO_4$ = bluish flocculent ppt. (copper albuminate).

(*c*) Add a little $AgNO_3$ = white ppt. (silver albuminate).

(*d*) Add a little $HgCl_2$ = white ppt. (mercury albuminate).

(*e*) To half an inch of egg-albumin solution in a test-tube add the same amount of ether, and shake vigorously. Let it stand a few minutes; notice that the albumin becomes coagulated. Repeat with serum albumin = no coagulation. N.B.—This test is rather troublesome.

(*f*) Fit the thermometer, by means of a perforated cork, into a test-tube containing one inch or so of undiluted white of egg, from I. Warm very gently in a large beaker full of water, and notice temperature of turbidity (about 59° C.), and of coagulation (about 63° C.).

IV. **Tests on the Yolk of an Egg.**—Place the yolk, saved from I., in a small evaporating dish. Break it and test as follows:

A. *Vitellin.*—Put about half an inch of yolk into a small test-tube and shake it well with three or four times that amount of ether. Let it settle, decant off the ether into a small evaporating dish; add more ether, shake, and decant again into the same dish. The whitish-yellow residue insoluble in ether, left in the test-tube, is impure vitellin. Let it dry for a few minutes in the steam of the water-bath. Shake a little of it with some water in

a test-tube. Notice that it does not dissolve. Mix all the rest of the vitellin with about three times its bulk of 10% salt solution, and warm gently in the water-bath. Notice that it makes a turbid, whitish solution. Test the solution, filtered if it seems very lumpy, as follows:

1st. Xantho-proteic test.

2d. Biuret test.

B. *Yolk Oil* (*Fatty matters, Lutein, etc.*).—Warm the ether solution in the evaporating dish, gently and carefully, over a hot water-bath, putting out the flame for fear of accidents. When the ether has evaporated, notice that a yellow oil is left. Put a drop or two of it in water in a test-tube and notice the globules of oil. With the rest in the dish mix a drop or two of HNO_3 conc., and notice that the yellow color changes to blue and is finally destroyed. Then add a drop or two of water and also of ammonic sulpho-cyanide, NH_4CNS; the reddish color shows iron.

LESSON IX.

CRYSTALLIN, MYOSIN, ACID AND ALKALI ALBUMIN.

CRYSTALLIN (GLOBULIN).

It is claimed by many that this proteid is identical with vitellin, described before. It occurs in the crystalline lens associated with, probably, other proteids of the same class, and also with minute quantities of lecithin, cholesterin, fats, and salts. It is prepared by extracting it with a weak solution of common salt, and by precipitating and reprecipitating it from this solution by either water or carbon dioxide. Its solutions coagulate at from 75° to 85° C.; they are not precipitated by saturating with NaCl, and are readily changed to acid and alkali albumins.

MYOSIN.

Formation.—This substance is formed by the coagulation after death of the muscle plasma, a process which causes the condition known as *rigor mortis*. The action is apparently similar to the formation of fibrin in the blood. The living muscular tissue contains a neutral or alkaline, yellowish, opalescent fluid, the muscle plasma. This spontaneously coagulates after death with more or less rapidity owing, perhaps, to the action of a myosin ferment upon a globulin body, myosinogen; and the slender threads and fibres of myosin, as they contract, press out a clear acid fluid, the muscle serum. The change is accompanied by heat and by the formation of lactic and other acids and of carbon dioxide. The muscle, after this coagulation has set in, becomes compact and rigid, and is shorter, thicker, and somewhat more dense and more opaque than before. The *rigor mortis* sets in in man at intervals after death varying from a few minutes to several hours; and it continues for a period varying from one to six or seven days, until relaxed by incipient decomposition.

Preparation.—Myosin is obtained by extracting it from muscular

tissue with a solution of either salt or ammonium chloride and is purified by precipitation in an excess of water.

Properties.—It dissolves readily in solutions containing 10% NaCl or 14 or 15% NH_4Cl, but is precipitated by an excess of salt. It dissolves in dilute alkalies without change, and with 3 or 4% hydrochloric or other acids it forms compounds known as acid myosins. By very weak hydrochloric acid (0.2%) it is converted into syntonin, from which it can be again produced by the action of lime and NH_4Cl. It coagulates partially at 40 to 45° C. and in flakes at about 50° C. It is readily attacked by both pepsin and trpysin, as is shown by the ready digestibility of meat.

PARAGLOBULIN.

(*Serum Globulin.*)

Occurrence.—This proteid occurs in blood serum associated with serum albumin, and probably to about the same extent, *i.e.*, from 3 to 5%. It can be separated either by diluting serum some eight or ten times with water and then thoroughly saturating it with carbon dioxide, or else by warming serum to 35° C. and saturating it with crystals of $MgSO_4$. The paraglobulin is then washed and purified by successive solution and precipitation.

Properties.—Paraglobulin dissolves most readily in NaCl solutions of a strength of 5 to 10%. In a solution containing less than 0.1% it is practically insoluble. It dissolves readily in dilute alkalies, from which it can be reprecipitated by CO_2 or by dilute acids. When held in solution by salt it coagulates at about 75° C. It sometimes occurs, associated with serum albumin, in pathological urines, but as the ordinary tests for the two proteids are identical and as there is no special significance that we know of in the presence of one rather than the other, we rarely take the trouble to distinguish them.

The tests on this proteid are given in Lesson XIX.

FIBRINOGEN.

Occurrence.—This name has been given to a substance occurring in the blood plasma, chyle, lymph, hydrocele fluid, and other coagulable fluids of the body, which, on contact with a little known fibrin ferment, is converted into fibrin, and thereby gives rise to the phenomenon of coagulation.

Preparation.—It can be obtained by letting blood run directly from the vessels into a solution of $MgSO_4$, then separating the corpuscles, and precipitating the fibrinogen by saturating the solution with NaCl. The precipitate is filtered off and dissolved in 8% NaCl solution, and is cleansed by reprecipitating and redissolving two or three times with saturated and with 8% NaCl solutions.

Properties.—The fibrinogen thus obtained dissolves completely in NaCl solution, and is readily precipitated by saturation with either NaCl or $MgSO_4$. If dissolved in a pure salt solution, it is also precipitated by carbon dioxide. It is coagulated, on heating to 55 or 56° C., to a substance not dissimilar to fibrin, and when this is filtered off there is found in the filtrate another globulin, which, however, is not the same as paraglobulin. If fibrinogen is heated for some time at about 38° C., it loses its power of coagulating both with heat and with the ferment. It is altered, by standing under water, until it no longer dissolves in salt solution. If all the alkali salts are dialyzed out, it loses almost entirely the power of coagulating to fibrin.

Uses.—Fibrinogen is exceedingly important in its relations to the clotting of the blood. The most generally received theory at present is that the fibrin is formed by the action on fibrinogen of a peculiar ferment, the fibrin ferment, which is spontaneously produced in blood, after death. Indeed certain recent observers claim to have produced a ferment which will coagulate fibrinogen and which they call protozym, by special treatment not only of white blood cells, but of leucocytes of the chyle, lymph, and other fluids, pus cells, the stroma of red blood cells, etc., etc. The fibrin produced is not equal in amount, it seems, to the fibrinogen present, and a second proteid is formed at the same time, which seems to be a globulin, but does not coagulate on heating.

Class III.—DERIVED PROTEIDS.

ACID ALBUMINS.

Preparation.—This class of proteids is derived from the albumins, globulins, coagulated proteids, and fibrin by treatment with mineral acids, dilute or concentrated, or with some metallic salts like ferric chloride, mercuric nitrate, and others.

Properties.—They differ more or less in their percentage composition, their action on polarized light, and their decomposition products. Indeed, it seems probable that for every member of the above classes there is at least one acid albumin to correspond. They all, however, have certain properties in common. They are insoluble in pure water and in neutral solutions, but readily dissolve in dilute acids. In diluted alkaline solutions they dissolve readily and are probably converted into alkali albumins. They do not coagulate on boiling nor with any ferment, and they represent the first step in the breaking down of the more complex proteids into the albumoses, peptones, and other products of digestion.

SYNTONIN.

Preparation.—This is the name given to the acid albumin prepared by digesting myosin with very weak (0.1 to 0.2%) hydrochloric acid. From the solution thus formed it can be precipitated by carefully neutralizing with dilute alkali.

Properties.—It is a pasty, whitish substance, insoluble in water and neutral solutions, but readily soluble in dilute acids or alkalies. It gradually loses its solubility in these reagents if allowed to stand some time under water. It can be precipitated from its acid solution by concentrated hydrochloric acid, or by strong solutions of NaCl, $MgSO_4$ and other neutral salts. It dissolves in lime-water, and while in this medium is partially coagulated by boiling, and indeed on further treatment with NH_4Cl possesses many if not all the properties of the original myosin. Its solutions both in acid and alkali, independent of their concentration, polarize to the left, $[a]_D$ being equal to $-72°$.

Uses.—It is of much importance as being the first stage in the action of the gastric juice, the proteids being probably converted into this substance before they are attacked by pepsin.

ALKALI ALBUMINS.

(*Albuminates.*)

Preparation.—These bodies are produced by treating the different varieties of proteids with alkali. They probably differ from each other, just like the acid albumins, according to their source

and methods of production. They were for a long time considered to be identical with the acid albumins, only dissolved in alkaline instead of acid media. This is now believed to be a mistake.

Properties.—When they are obtained in a pure state, by neutralizing the alkaline solution with dilute acid, and dissolving and reprecipitating the precipitate thus formed, they are found to have some distinct chemical properties. They dissolve very slightly in water, in common salt solutions, and also in weak lime water, with an acid reaction. They form metallic compounds when mixed with barium, strontium, and calcium carbonates, and set free carbon dioxide. Even an excess of mineral acid does not readily change them into acid albumin, although it readily dissolves them when freshly precipitated. They polarize strongly to the left, the angle, in the case of those formed from serum albumin and a strong solution of caustic potash, being as large as $-86°$.

LABORATORY EXPERIMENTS.

CRYSTALLIN, MYOSIN, ACID AND ALKALI ALBUMINS.

I. **Crystallin (Globulin).**—Extract the crystalline lens from the eye of an ox, crush it in a mortar, and rub it up with a little ½% salt solution. Filter it, and test the filtrate for crystallin.

1st. Biuret test.

2d. Millon's test.

3d. Ferrocyanide test.

4th. Put the solution in a test-tube, insert thermometer as in Lesson VIII., warm very gently in large beaker full of water, and notice temperature of coagulation (above 75° C.). N.B.—This solution will coagulate in flakes, and not, like the white of egg, in a solid mass.

II. **Myosin.**—Soak chopped muscle in water for fifteen minutes, strain and squeeze out the juice through wet muslin, and test this liquid for soluble proteids (serum albumin, etc.) by some general proteid reactions and by heating it. Then soak and squeeze out the muscle in water four times, rub up the washed

muscle in a mortar with 10% salt solution, strain through muslin, filter if necessary, and test liquid for *myosin*.

1st. Biuret test.

2d. Millon's test.

3d. Fill a beaker with water and add just one drop of solution; notice the ppt.

4th. Put in a beaker one inch of dry NaCl and two inches of water. Shake and warm till the salt is nearly dissolved; let it cool and settle; decant off the clear liquid into another beaker, and to it add one drop of the solution; notice the ppt.

III. **Acid Albumin.**—Dissolve some egg albumin in water in a test-tube, add some HNO_3 conc., boil, and filter if necessary.

Test the solution (of acid albumin) as follows:

1st. Boil it = no coagulation.

2d. Put some in a test-tube, add a little piece of litmus paper, and neutralize it very carefully with KOH, using at first some KOH from the bottle, and for the final additions using some of that KOH much diluted with water. The test-paper will show when the acid is nearly neutralized. When the liquid is just neutral, there will be a ppt. of acid albumin.

3d. To this ppt. add a little more KOH; it dissolves at once.

IV. **Alkali Albumin (Albuminate).**—Make a saturated solution of egg albumin in an evaporating dish, with some water, and add, drop by drop, with constant stirring, six or eight drops of a saturated NaOH solution. Notice in a minute or two that the alkali albumin forms in gelatinous masses through the liquid. Dissolve it in some more water and test the solution as follows:

1st. Boil it = no coagulation.

2d. Neutralize some very carefully with diluted HCl, and exactly at the neutral point there will be a ppt.

3d. To this ppt. add a little more acid; it dissolves at once.

N.B.—To get satisfactory results with the neutralizing experiments, great care and delicacy are required.

LESSON X.

FIBRIN, COAGULATED PROTEIDS, LARDACEIN—THE VEGETABLE PROTEIDS—TESTS FOR SULPHUR IN PROTEIDS.

Class IV.—FIBRIN.

Formation.—This is produced by the clotting of blood, lymph, chyle, and other coagulable fluids of the body. It generally forms very soon after the shedding of blood from the vessels, and its presence is of the utmost importance in checking the flow of blood from wounds, which, even if of slight extent, it would be otherwise almost impossible to stanch. It is formed, as before stated, by the action of the fibrin ferment on fibrinogen. As it forms it first spreads through the liquid in the form of innumerable microscopic threads radiating in every direction, and forming a thin jelly-like mass. This gradually stiffens and the fibres become thicker and more firm, until the mass is solid, and can be inverted, even in bulk, without disintegrating. Then the fibres begin to contract and, retaining the corpuscles in their meshes, gradually squeeze out more or less of a clear, thin fluid, the blood serum.

Preparation.—We can obtain fibrin in quite a pure state by whipping or, better still, kneading the fibres out of a bowl of coagulating blood, and washing and working them in water until no longer colored.

Properties.—The fibrin thus obtained is a tough, white, stringy, elastic, fibrous material. If heated in water or other neutral liquids to 72° C., it loses its elasticity almost entirely and becomes more like threads of coagulated egg or serum albumin. It dries to brown, brittle, irregular, shrivelled-up masses, which can be powdered in a mortar. These masses can be restored to almost their original condition by soaking in water. It is wholly insoluble in water or alcohol, but, when kept in the former, de-

composes, and in the latter becomes with time thick and less elastic. It dissolves readily in diluted alkalies to an alkali albumin, swelling first and becoming transparent. It swells very readily in dilute acids, hydrochloric, acetic, and others, changing to a translucent, gelatinous mass, even in the cold. On warming, it dissolves to acid albumin. It oxidizes slowly in the air; when fresh and moist, and especially in a pure atmosphere of oxygen, it absorbs that gas and sets free carbon dioxide. It also decomposes a solution of hydrogen peroxide, liberating oxygen. Neither of these properties is possessed by the boiled fibrin, nor is the latter as readily attacked by dilute hydrochloric acid and pepsin, as when fresh.

The tests on this proteid occur in Lesson XIX., under the Blood.

Class V.—COAGULATED PROTEIDS.

Formation.—These substances are produced by the action of heat, electricity, mineral acids, alcohol, and other reagents upon proteids of the preceding classes. It seems probable that, just as in the case of the derived proteids mentioned before, there is a separate coagulated proteid corresponding to every individual albumin, globulin, and the rest; but our knowledge of them is as yet very limited. We believe that their formation depends more or less upon the presence of certain mineral salts, especially the phosphates of the alkaline metals; but their exact relations with these substances are not known.

Properties.—They all agree in being insoluble in water and in solutions of neutral salts, and in dissolving with difficulty in dilute acids and alkalies. They are changed by strong caustic alkalies and by warm acids, to alkali and acid albumins respectively. They readily undergo both gastric and pancreatic digestion.

Class VI.—AMYLOID SUBSTANCE—LARDACEIN.

Occurrence—This is a very peculiar substance, which is sometimes found in severe wasting diseases as a degeneration product of the walls of the blood-vessels in the spleen, liver, kidneys, and other organs. It gives the tissues a peculiar translucent appearance.

Preparation.—It is obtained from the tissues containing it by a process of elimination. The material is thoroughly extracted with water, hot and cold, then with alcohol, ether, dilute acids, and other solvents, and finally what remains is digested with artificial gastric juice. The amyloid substance remains unchanged and can then be separated mechanically from the small residue.

Properties.—Thus prepared it appears as a white powder, insoluble in any of the above media, but changed by concentrated hydrochloric acid to acid albumin, and by caustic alkalies to albuminate. It differs from the coagulated proteids as well as from all the before-mentioned albuminous bodies in not being attacked by pepsin and hydrochloric acid, and also by being colored red by iodine. If treated first with strong sulphuric acid and then with iodine, its color changes to violet, and even to a purplish blue. It resembles the carbohydrate group, however, in no other respect, and its products of decomposition are distinctly those belonging to a proteid.

The discussion of Classes VII. and VIII., the Albumoses and the Peptones, will be left until Lesson XXI., on Digestion.

VEGETABLE PROTEIDS.

Occurrence.—Although all proteids are formed originally in the vegetable kingdom, and are only metamorphosed by animals, they are not as widely scattered in plants as we might expect. They are principally stored up, much as starch is, in the seeds, roots and tubers in the form of minute granules (protein grains), of varying shapes and sizes, and covered with a coating of more insoluble material. The albuminous bodies contained therein differ from those hitherto described, by not infrequently occurring in the form of clear, well-defined crystals. It has been thought probable that these forms are combinations of the proteids with some metallic bases, but the latest analyses do not tend to confirm this theory.

Properties.—The vegetable proteids are quite similar to those belonging to the previous classes, both in the general reactions, the action of heat and various reagents, the effect of the gastric and pancreatic ferments, and the products of decomposition. It is important to remember, though, that, however similar their

properties may be, we have not yet found a single albuminous body common to both plants and animals. So we conclude that the vegetable proteids are decomposed in the process of digestion, and that their constituents are reunited again to form similar but not identical compounds.

Classification.—They have been arranged, roughly, in classes more or less corresponding to those we have just discussed. Their properties, however, do not always agree, and the classification on the whole is a most unsatisfactory one. There is one class, indeed, that of the glutin compounds, which has no counterpart among the animal proteids, and on these we have made some few tests.

CLASS I. **Plant Albumins.**

It is rather doubtful whether any of the vegetable proteids can be properly considered as coming under this head. To be sure, it is possible to make aqueous extracts of certain plant tissues, and then, after carefully neutralizing, to coagulate the solution by heat. But it is still doubtful whether the albuminous bodies thus extracted would have been dissolved if it were not for the presence of minute quantities of neutral salts. In other words they may, on careful investigation, have to be classed as globulins, and not albumins.

The different substances that are generally included under this head can be soaked out in small quantities by warm water from the various kinds of flour, and from pounded peas, beans, and other seeds. They seem to answer most of the tests common to egg or serum albumin.

CLASS II. **Plant Globulins.**

These form a large class of compounds, and occur in certain seeds, like hemp, castor-oil bean and others, and also in various nuts, Para nuts, walnuts, hazelnuts, sweet and bitter almonds, etc.

These are first extracted with ether and other solvents to remove the oil and fat, and then the proteids are dissolved out with dilute alkalies or with salt solutions.

They resemble the animal globulins, in dissolving in salt solutions and coagulating on heating. But in some cases they dissolve more or less in pure water, and can be precipitated from

this solution by a little salt, and then redissolved in more concentrated NaCl solutions. Some of them, also, are found in the form of crystals and can even be crystallized artificially from their solutions.

CLASS III. **Plant Caseins.**

These are soluble in dilute alkalies, and are precipitated from their solutions by neutralizing with acid.

The most important member of this group is legumin, which is found in abundance in oats, peas, beans, lentils, and similar vegetables. It can be extracted with weak alkalies, it is insoluble in water, and is coagulated on boiling. It is of great value as a food stuff.

Another member of this group is the so-called gluten casein, which differs from the other gluten compounds in dissolving but slightly in alcohol. It dissolves readily in very dilute alkali, and forms metallic compounds on adding cupric sulphate or other salts to this solution.

CLASS IV. **Gluten Proteids.**

Occurrence.—Gluten is a peculiar albuminous material found in wheat, rye, and other cereals, and which is of the utmost importance in the manufacture of bread. It is not dissolved by water, but, when moistened, forms a sticky elastic mass, and makes the dough tough and tenacious. So when the bread is raised by means of carbon dioxide or in some cases by air, the bubbles of gas expand the dough, and make it spongy, instead of passing right through it as would be the case if starch and water only were present. Then, too, on baking, the gluten is partially coagulated, becoming firm enough to hold its shape, and not shrink back to its original condition. In this way the finished loaf, instead of being hard and compact, is light and friable, and hence easily masticated and digested.

Preparation.—The gluten can be extracted from flour, preferably good wheat flour, by first making a lump of stiff dough, and then gradually working and kneading this dough so that the starch strains out and the sticky, pasty material is concentrated. If too much water is mixed with the flour at the start, the gluten cannot be separated, and the lump of dough must be kept intact while working, or the little particles of gluten will be washed

away. It is sometimes easier to wrap the lump of dough, before working it in the water, in a piece of muslin or cheese-cloth, which will let the starch granules through, but keep back the little lumps of gluten. But with care it can be extracted just as well by hand.

Composition.—The gluten thus obtained is not very pure. It generally contains, besides the albuminous matter, small amounts of starch, fats, salts, and other substances. It dries to a hard, brownish, horny mass. It is insoluble in cold water, slightly soluble in boiling water, and insoluble in neutral salt solutions and in absolute alcohol. It dissolves more or less easily in dilute acids and alkalies, and also to a considerable extent in hot dilute alcohol. It is claimed by some authors that it does not exist as such in the dry flour, but is produced, on the addition of water, from some pre-existing globulin body, much in the same manner that fibrin and myosin are produced.

The crude gluten from wheat flour seems to consist of at least four distinct proteids, which can be separated with some difficulty according to their solubility in alcohol and other reagents.

These are—

1st. *Gluten casein*, comparatively insoluble in hot alcohol.

2d. *Gluten fibrin.*

3d. *Gliadin* or *Plant gelatin.*

4th. *Mucedin.*

The last three are soluble in hot alcohol more or less diluted. They do not present any special points of interest.

SULPHUR IN PROTEIDS.

The different reactions which are included under this heading are important, not only as applying to the various albuminous bodies, but also in testing for unoxidized sulphur compounds, both organic and inorganic, as in mineral waters, sewer gas, and so forth.

1st. **The Lead Test.**—This is the simplest and most satisfactory test that we have. It depends upon the formation of black insoluble plumbic sulphide whenever unoxidized sulphur meets an alkaline solution containing lead. The solution most readily obtained is an alkaline solution of plumbic hydrate, and is formed

milky with starch. Pour off the water carefully, add fresh water, and continue working the lump of dough until no more starch comes out. The sticky elastic residue is *gluten*. Test it as follows:

1st. With the xantho-proteic and Millon's tests.

2d. Boil it with water; notice that it neither dissolves much nor hardens perceptibly.

3d. Boil it in a test-tube with water and one drop of KOH; notice that it decomposes and dissolves to some extent. Pour some of the solution into another test-tube and add a little $CuSO_4$ = ppt. which does not dissolve on the addition of a little HCl dil.

4th. Shake some thoroughly in a test-tube with alcohol diluted with a few drops of water, warming it carefully in the water-bath. Test the liquid for dissolved gluten proteids by the biuret test.

N.B. These last two tests on gluten are not very satisfactory unless done with great care and some patience.

IV. **Sulphur in Proteids and Albuminoids.**.—Test egg albumin for sulphur as follows:

1st. *Lead Test.*—Put in a test-tube one inch of $Pb(C_2H_3O_2)_2$; heat it, and add to it one inch of KOH. Notice the white ppt. of $Pb(OH)_2$. Continue to add KOH, heating all the time, until this white ppt. has almost or entirely dissolved. Add some of this clear solution to a test-tube full of water, boil it, and, as it boils, add a little dry egg albumin = *black*, from formation of PbS. Repeat this test with solutions of egg albumin, more and more dilute.

2d. *Bismuth Test.*—Boil in a test-tube some water containing several drops of Nylander's solution, and, as it boils, add some dry egg albumin, and also some solutions of albumin, more or less dilute. Notice the changes of color, and observe that this reaction will interfere with the test for glucose in Lesson II.

3d. *Sodic Nitro prusside Test.*—Powder some dry albumin in a mortar. Moisten the platinum wire with water and dip it in this albumin; then moisten it again and dip it in some dry Na_2CO_3 so as to cover the albumin as far as possible. Fuse the mixture on the wire very gently in a *smoky* flame, and dissolve the bead in

drop of water in an evaporating dish. To it add another drop of water in which has been dissolved a single small crystal of sodic nitro-prusside. Notice the purple color where the two liquids meet.

Try to repeat this test, using a single hair instead of the powdered albumin.





PART IV.

THE INORGANIC CONSTITUENTS OF THE BODY.



LESSON XI.

OXYGEN, HYDROGEN, CHLORINE, AND HYDROCHLORIC ACID.

It is not proposed in the next five lessons to give the student any regular course in qualitative analysis, but simply to review, briefly, some of the more prominent features and tests of the acids and bases occurring most frequently in the body.

OXYGEN.—O_2.

Atomic Weight = 16.

History.—Discovered by Priestley, 1774, and by Scheele, 1775; named by Lavoisier, who thought that it could make acids.

Occurrence.—Most abundant element in nature. Occurs free in the atmosphere (23% by weight). Combined it is found in gases, *e.g.*, CO_2, and SO_2; in liquids, *e.g.*, water, 88.9%, alcohol, ether, and oxygen acids; and in solids, *e.g.*, all ordinary rocks and many minerals. It forms nearly 50% by weight of the earth's crust. It is contained more or less abundantly in almost all organic compounds except the hydrocarbons, and in every part of animals and plants.

Preparation.—By heating metallic oxides, HgO, MnO_2, BaO_2, and others. Also by heating $KClO_3$, which decomposes into KCl and free oxygen, according to the formula

$$2KClO_3 \text{ heated} = 3O_2 + 2KCl.$$

The gas can be collected over water.

Properties.—(*a*) *Physical.*—Oxygen is a colorless, odorless, tasteless gas, exceedingly difficult to liquefy. It is 1.1 times heavier than air, and dissolves in water to the extent of 3 or 4%.

Upon this last property depends the life of aquatic plants and animals.

(*b*) *Chemical.*—It combines more or less readily with every

known element except fluorine, forming oxides and oxygen salts. This combination is always accompanied by more or less heat, and is called combustion when both heat and light are evolved.

It is the active agent in the atmosphere, and, through its property of oxidizing organic substances, is the great source of energy and life. Pure oxygen acts more energetically than the atmosphere, which is diluted with four times its weight of inert nitrogen. Hence various substances burn in O_2 which do not burn in air. In the combustion of organic bodies both in and out of the body it converts carbon into CO_2 and hydrogen into H_2O. The nitrogen in the body is oxidized to urea, $(NH_2)_2CO$, and its allies.

Many oxides formed from non-metallic elements form acids on the addition of water. Thus, $CO_2 + H_2O = H_2CO_3$, carbonic acid, which forms true salts with metallic and other bases. Thus, with limewater:

$$\underset{\text{Limewater}}{H_2CO_3 + Ca(OH)_2} = \underset{\text{Calcium Carbonate}}{CaCO_3} + 2H_2O$$

The oxides of the metals are either neutral or, if of the alkaline and alkaline earthy metals, alkaline bases.

(c) *Physiological.*—Oxygen is the source of energy and life for both plants and animals. In plants, however, the energy needed is comparatively slight.

Hence, in them, but little oxygen is consumed compared to that set free by the building up of the carbohydrates from CO_2 and water.

Uses.—This element is used pure to some slight extent in medicine, in disorders of respiration and circulation, and in cases of carbonic-oxide poisoning. Also in the arts, for the oxyhydrogen blowpipe and light, for purifying gas, for the preparation of varnishes, of metallic and other paints, and for numerous less important purposes.

HYDROGEN.—H_2.

Atomic Weight = 1.

History.—Hydrogen was observed by Paracelsus in the sixteenth century, and was carefully investigated in 1766 by Cavendish, who later proved that it formed water on combination with oxygen. It was named after this property by Lavoisier.

Occurrence —It occurs free, in small quantities, 'occluded' in meteorites; also in volcanic and other natural gases, and as one of the products of decomposition in sewer, intestinal, and other gases. In combination it is found in the atmosphere in the form of ammonia, nitrous and nitric acid, and marsh gas. It forms 11% by weight of water in all forms, and is an essential constituent of all acids and acid salts, and most organic compounds.

Preparation.—Hydrogen can be prepared from water by electrolysis or by removing the oxygen with some metal, such as sodium in the cold, or iron at a high temperature. It is also produced when an acid like H_2SO_4 or HCl is allowed to act upon a metal, in which case the latter replaces the hydrogen of the acid and forms a salt. The energy derived from this decomposition is set free, partly as heat, but also in the form of electricity, and the reaction proceeds with much more freedom if an electro negative inert metal, like copper, is mixed with the zinc or iron which is to be dissolved, thereby forming a true voltaic couple. The reaction is simple :

$$Zn + H_2SO_4 = ZnSO_4 + H_2.$$

The gas produced in this way is rarely quite pure and is usually washed in dilute alkalies and water.

Properties.—(*a*) *Physical.*—Hydrogen is a colorless, odorless, tasteless gas ; it is the lightest and the most diffusible substance known, and is the most difficult of all gases to liquefy.

(*b*) *Chemical.*—While this element does not support ordinary combustion, it burns readily with a pale blue flame in either air or oxygen, forming water. Mixed with air or oxygen it is extremely explosive, and hence must be handled with great care. It acts as a rather powerful reducing agent.

When hydrogen is evolved in the presence of soluble salts of arsenic, a colorless, unpleasant-smelling, very poisonous gas is produced called arseniuretted hydrogen, AsH_3. This substance burns readily in the air, being converted into water and arsenious oxide, which latter emits a garlic odor and colors the flame bluish-white. On heating the gas in a tube, or on cutting off the supply of air from the burning jet, it is decomposed into hydrogen and metallic arsenic, which latter deposits as a black mirror on any adjacent cool surface.

Hydrogen acts with compounds of the metal antimony in just the same way, but the arsenic gas can be distinguished from the antimoniuretted hydrogen, on passing it into a solution of argentic nitrate. A black precipitate is formed in each case; but any arsenic that is present will be dissolved in the liquid, and, after filtering, can be tested for in the filtrate by ammonic hydrate. The antimony, however, combines with the silver to form a black, insoluble, argentic antimonide. The arsenic mirror can also be distinguished from the similar deposit of antimony by dissolving readily in a drop of nitric acid, and then, on evaporation, giving a brick-red stain of argentic arsenate, Ag_3AsO_4, when touched with a drop of argentic nitrate.

This test, known as Marsh's test, is in many respects the most delicate and satisfactory of all the tests for arsenic.

Uses.—Pure hydrogen is rarely used as a reagent; it is sometimes employed for the oxyhydrogen blowpipe, though for this purpose it is generally replaced by coal gas. It has also been employed for balloons, but without much success on account of its great diffusive power.

Mixed with carbonic oxide it is largely used, in the form of water gas, for the production of both heat and light.

CHLORINE.—Cl_2.

Atomic Weight = 35.4.

History.—This gas was discovered by Scheele in 1774, and investigated and named after its color by Davy in 1810.

Occurrence.—Chlorine never occurs free in nature, but its compounds are found in large quantities and almost universally disseminated. In the form, principally, of sodium chloride, it occurs to a slight extent in the atmosphere; it is present in all natural waters, especially in sea and certain mineral waters, and in all soils. Great deposits of common salt, and sometimes of chlorides of potassium and magnesium, occur in different parts of the world, and chlorides of ammonium, silver, copper, and other metals are among the rarer minerals. The chlorides, generally of the alkaline metals, are almost always present in the juices of both animals and plants, although usually in but small quantities.

Preparation.—Chlorine is usually prepared by removing the

hydrogen from hydrochloric acid with the aid of binoxide of manganese. The hydrogen is oxidized and converted into water, while part of the chlorine combines with the metal, and the rest is set free. Thus:

$$MnO_2 + 4HCl = Cl_2 + \underset{\text{Manganous chloride}}{MnCl_2} + 2H_2O$$

For uses in the arts, this chlorine, after preparation, is stored up in a portable form by passing it over slaked lime and converting it into bleaching powder, $CaOCl_2$. From this compound chlorine is set free, on the addition of acid, according to the formula,

$$CaOCl_2 + 2HCl = Cl_2 + CaCl_2 + H_2O,$$

or, if sulphuric acid were used,

$$CaOCl_2 + H_2SO_4 = Cl_2 + CaSO_4 + H_2O.$$

Properties.—(*a*) *Physical.*—Chlorine is a greenish-yellow gas with an intensely suffocating odor. It is nearly two and a half times heavier than air, and can be condensed, without much difficulty, into a yellow liquid. It readily dissolves in cold water, and the solution possesses properties similar to those of the gas.

(*b*) *Chemical.*—Chlorine does not burn in or unite very readily with oxygen. It burns, however, in hydrogen, and unites with it so readily to form HCl that it can take it out of comparatively stable compounds.

It acts upon organic compounds in three different ways; it either combines with them directly, or it substitutes itself for one or more hydrogen atoms, or, in the presence of water, it combines with the hydrogen of the latter and liberates oxygen, which latter, being in a nascent state, is exceedingly active, and oxidizes readily anything that it is in contact with. Hence chlorine is a powerful bleaching agent and deodorizer, and in some cases acts as a disinfectant, and it is exceedingly corrosive to all delicate organic tissues.

Chlorine unites readily with the metals, especially if, as in aqua regia, it is in a nascent state. These metallic chlorides are always neutral, and are usually soluble in water. Silver and mercurous chlorides are exceptions to this rule, and hence serve as tests for the presence of chlorine or of soluble chlorides.

(c) *Physiological.*—This element is exceedingly irritating to the mucous membranes, and may even produce a corrosive effect. Inhaled in minute quantities, it acts as a stimulant, but in larger quantities it causes inflammation of the mouth, throat, nose, and other respiratory passages, accompanied with coughing and in severe cases with suffocation. Plenty of fresh air is the most important remedy, but it is possible to neutralize its effects somewhat by the use of ammonic hydrate, both for inhalation, and, in very dilute solutions, for gargling and swallowing.

Uses.—Chlorine is used enormously for bleaching purposes, almost always in the form of bleaching powder. It also enters into the manufacture of certain important compounds like chloroform, and the chlorates of potash and soda. As a deodorizer it is valuable, but when used for disinfection it has two serious disadvantages: it does not destroy all the germs, and it does tarnish, bleach, and corrode all metallic or colored surfaces that are exposed to it.

HYDROCHLORIC ACID.—HCl.

History.—This substance was known to the alchemists, both pure and in the form of aqua regia. Priestley first obtained the pure gas, collecting it over mercury instead of water, and its composition was determined by Davy in 1810.

Occurrence.—Hydrochloric acid gas is found in the vapors from volcanoes, and also, in minute quantities, in the atmosphere near chemical works. In solution it is sometimes found in rivers and streams in volcanic regions. It occurs in small quantities in the gastric juice of the higher animals.

Preparation.—It can be directly produced by the action of chlorine on hydrogen or its compounds. In practice, however, it is always prepared by treating common salt with sulphuric acid, when the sodium of the salt takes the place of the hydrogen of the acid. According to the comparative quantities of salt and acid used, we have the following reactions:

$$NaCl + H_2SO_4 = HCl + NaHSO_4 : \text{sodic bisulphate;}$$

or $$2NaCl + H_2SO_4 = 2HCl + Na_2SO_4 : \text{sodic sulphate.}$$

This is done on an enormous scale in the preparation of soda from common salt by the Leblanc process.

The crude hydrochloric acid, which is obtained as a by-product in the soda industry, is more or less contaminated with iron, sulphuric acid, and other impurities, but can be easily purified by distillation.

Properties.—(*a*) *Physical.*—True hydrochloric acid, HCl, is a colorless gas, with a suffocating acid smell and taste. It is 1.28 times heavier than the air, and can be liquefied without much difficulty. It is neither inflammable nor does it support combustion. It is exceedingly soluble in water, one part of water at 0° C. dissolving 503 volumes or 82% by weight of the pure gas. This solution, the hydrochloric or muriatic acid of commerce, is a colorless liquid, fuming in the air when strong, and smelling strongly of the gas.

(*b*) *Chemical.*—This acid forms with bases and metals the same compounds as chlorine. When brought into the presence of ammonic hydrate, it combines with the ammonia to form white fumes of ammonic chloride. This reaction serves as a test both for hydrochloric acid and for ammonia. The most delicate test for this acid, as well as for all soluble chlorides, is the formation of argentic chloride when it is mixed with a soluble salt of silver in the presence of nitric acid. Silver chloride is insoluble in water and dilute acids, but dissolves readily in ammonic hydrate. It is acted on by light, and hence is largely used in photography. It is decomposed into metallic silver by zinc or some similar metals according to the following reaction:

$$2AgCl + Zn = Ag_2 + ZnCl_2.$$

Another, but not very delicate, test is the formation of plumbic chloride by the action of the acid or its salts upon a soluble salt of lead. This compound is somewhat soluble in water and in concentrated hydrochloric acid, but is not so soluble in dilute acid. It is not dissolved by ammonia. In both these cases the precipitate is formed by the simple replacing of hydrogen by a metal. Thus:

$$AgNO_3 + HCl = AgCl + HNO_3$$

and

$$\underset{\text{Plumbic Acetate}}{Pb(C_2H_3O_2)_2} + 2HCl = PbCl_2 + \underset{\text{Acetic Acid}}{2HC_2H_3O_2}.$$

If the acid acts upon a metal directly, then the hydrogen is liberated. Thus:

$$Zn + 2HCl = ZnCl_2 + H_2.$$

(*c*) *Physiological.*—Hydrochloric acid plays a very important part in the digestion of proteids, its presence being essential to the best action of pepsin. The strength of this acid in the gastric juice seems to be from 0.1 to 0.2%. In very small quantities it is often administered in dyspepsia and other disorders of digestion, even where the stomach is extremely acid from different organic acids, and is found of considerable value. When concentrated, it acts as an irritant poison and must be counteracted by copious dilution with water and the administration of mild alkalies like bicarbonate of soda, and of emollients.

Uses.—Most of the hydrochloric acid produced is used for the manufacture of bleaching powder. It is, besides this, a most valuable reagent, and is employed not only in the laboratory but also in a large number of chemical processes.

LABORATORY EXPERIMENTS.

OXYGEN, HYDROGEN, CHLORINE, AND HYDROCHLORIC ACID.

I. **Oxygen.**—O_2.—N.B. The oxygen experiments should be made by two men working together.

(*a*) *Preparation.*—Mix potassic chlorate, $KClO_3$, with an equal amount of dry sand, and with the mixture almost fill a large test-tube. Fit to this a delivery-tube, the same as used in Lesson IV., and heat gently and gradually. Fill the saucepan two-thirds full of water, and in this hold a wide-mouthed bottle, inverted, full of water. (This bottle should be filled quite full, and then a plate of ground glass should be pressed against the mouth while it is being inverted. On putting its mouth under the level of the water, the bottle is kept full.) Wait for a few minutes until the $KClO_3$ is evidently beginning to fuse, and then pass the gas through the water into the inverted bottle. When all but half

an inch of water has been replaced by the oxygen, remove the delivery-tube and the flame, press the ground glass against the mouth of the bottle, and place the latter upright on the table.

(*b*) *Tests.*—The gas, unless impure, will be colorless and tasteless. To test its combining power, fasten a fragment of charcoal to a piece of wire, and heat it till part of it is red hot; then take off the glass plate and put the charcoal inside the bottle. It will glow brightly, and even burn. When the combustion has ceased, remove the charcoal, put on the glass plate, and shake the water in the bottle vigorously up and down for a minute or two. Then test this water—

1st. With a piece of litmus paper = very faintly acid from carbonic acid.

2d. Put it in a test-tube and add a few drops of $Ca(OH)_2$ = very faint white ppt. of $CaCO_3$.

II. **Hydrogen.**—H_2.

(*a*) *Preparation.*—Place the zinc turnings in the flat-bottomed thick glass evolution flask, and fill it one-third full of water. To the end of the exit-tube attach, with a piece of rubber tubing, the small ignition-tube of hard glass. Then add about half an inch of H_2SO_4 dil. and notice the evolution of H_2 gas.

(*b*) *Tests.*—Notice that the gas is colorless and almost odorless. When the action diminishes add a few drops of acid, and, after it has run well for fully five minutes, wrap a towel around the flask, and ignite the stream of gas issuing from the ignition tube. Notice that it burns at first with a blue, but after the glass gets heated, with a yellow or reddish flame. Wipe the mortar dry and clean and hold it for a minute against the burning jet, moving it round a little. Notice that the porcelain is not discolored, and that small drops of *water* collect on its surface.

Marsh's Test for Arsenic.—Now add to the flask, without disconnecting or altering anything, a few drops of a solution of white arsenic, As_2O_3, in HCl dil. Notice that the color of the flame changes almost at once to white or gray. Then place the porcelain mortar against the burning jet, and there will be deposited a black mirror of metallic arsenic. Dissolve this mirror in a drop of HNO_3 conc.; dry it gently over the flame, and then

touch the spot with a drop of $AgNO_3$. Notice the red stain of Ag_3AsO_4.

Also heat with the burner the glass ignition-tube three or four inches from the rubber connection, and a similar mirror will be deposited inside the tube.

III. **Chlorine.**—Cl_2.—N.B. Great care must be taken not to inhale this gas.

(*a*) Place the bleaching-powder, $CaOCl_2$, in a thin glass flask, moisten it with water, and fill the flask one-third full of HCl dil. Fit to it at once the delivery-tube, warm gently, and pass the yellow Cl gas into a beaker or flask of water. Notice the color and smell of the gas and of the water solution, and test the bleaching properties of both gas and solution by holding in them various test papers, and by adding them to indigo and fuchsin in test-tubes.

(*b*) N.B.—This experiment may be omitted, if pressed for time.

Place in a similar flask the manganese dioxide, MnO_2, and about half an inch of HCl conc. Heat carefully and pass the gas into water as above. Notice the same color, smell, and bleaching properties; also moisten a piece of copper and expose it to the gas. Notice that the Cl_2 tarnishes it.

IV. **Hydrochloric Acid.**—HCl.

Place in a flask about half an inch of dry NaCl, fill it one-third full of H_2SO_4 dil., heat it till it boils hard, and pass the gas into water in a beaker. Notice the great solubility of this gas in water. Test the solution as follows:

1st. Taste and smell it.

2d. Notice that it is acid to test paper.

3d. Pour a few drops of NH_4OH on a piece of filter paper and expose to the gas = white fumes of NH_4Cl.

4th. Put some solution in a test-tube, add a drop of HNO_3 dil. and a few drops of $AgNO_3$ = white curdy ppt. of AgCl. This ppt. dissolves in an excess of NH_4OH, and is reprecipitated by acids. It turns violet on standing in the light. Filter off some of this ppt. and place it on a watch-glass, with a small piece of zinc and a drop of water. Notice how the AgCl changes to black, spongy, metallic silver.

5th. To some of the solution in a test-tube add a little Pb



acetate = white ppt. of $PbCl_2$. To this add NH_4OH; notice that the ppt. does not dissolve.

6th. Pour some of the solution on a piece of zinc in a test-tube; notice the evolution of hydrogen gas.

N.B.—If the last two tests do not work satisfactorily, repeat them with HCl dil. from the shelf.

LESSON XII.

SULPHURIC, CARBONIC, AND NITRIC ACIDS.

SULPHURIC ACID.—H_2SO_4.

History.—The early alchemists were acquainted with this acid, which they called oil of vitriol, and which they obtained in small quantities by the distillation of green vitriol, $FeSO_4$. It has only been prepared and used on a large scale in the last hundred years or so.

Occurrence.—It is occasionally found free in river and spring waters, where it comes from the spontaneous decomposition of certain mineral sulphates. It is also sometimes found in the atmosphere, derived from chemical or metallurgical establishments. Its salts are numerous and widely scattered. Thus in sea water and in mineral springs we find sulphates of lime, magnesia, soda, and potash, and among the common minerals are gypsum, anhydrite, barite, celestine, and many others, compounds of this acid with lime, baryta, strontia, and other bases.

Preparation.—Small quantities of the fuming acid are still prepared by distillation of ferrous sulphate, but all the acid of commerce is derived from the oxidation of sulphur dioxide by the aid of the oxides of nitrogen. The operation is conducted in large leaden chambers, and the weak acid resulting is concentrated by evaporation

Properties.—(*a*) *Physical.*—Concentrated sulphuric acid is a heavy, oily liquid, of a specific gravity of about 1.84. When pure it is colorless and with little or no odor, but, even when very much diluted, with a strong acid taste. It boils at 338° C., giving white corrosive vapors, which on condensation still contain about 2% of water. It does not evaporate at any ordinary temperature. It is exceedingly hygroscopic, absorbing water with great avidity, not only from the atmosphere, but from compounds containing it.

When mixed with water, a diminution of volume results, and great heat is evolved. This seems due to the formation by the acid of definite chemical compounds with water.

(*b*) *Chemical.*—On all organic bodies sulphuric acid when concentrated acts as a powerful dehydrating agent, taking out the elements H and O in the proportions to form water. This is notably the case with the carbohydrates, wood, cotton, sugar, etc., where the abstraction of water leaves only caramel-like bodies or even carbon itself behind. When allowed to act momentarily on paper it produces the peculiar substance, parchment paper (v. Cellulose), which for all practical purposes is now used to replace dried animal membranes. If the action is continued more than a few seconds, however, the paper is destroyed.

On metals in general, strong sulphuric acid in the cold acts but slightly. When heated, it converts many of them, Cu, Pb, Hg, and others, into the corresponding sulphates, with evolution of SO_2 gas. When diluted, it readily attacks many of the metals, Fe, Zn, Mn, and others, setting free hydrogen. The sulphates thus produced are generally soluble, but there are two or three which are sufficiently insoluble in the ordinary media to serve as good tests for the presence of the acid. The best of these is $BaSO_4$, formed whenever sulphuric acid or a soluble sulphate is mixed with a soluble salt of Ba. It is a white, heavy precipitate, exceedingly insoluble in water, acids, and alkalies, and hence is a most delicate test. The calcic sulphate is much more soluble in water and dilute acid, and is less valuable as a test. The plumbic sulphate is very insoluble, but is more valuable as a test for lead than for sulphuric acid. It explains the reason why sulphuric acid or soluble sulphates like Epsom or Glauber's salts are such good antidotes to lead poisoning.

(*c*) *Physiological.*—Sulphuric acid itself has not been found in man, but its salts are present everywhere, though less in quantity than the chlorides. It is extremely corrosive to all the tissues of the body, and hence is not uncommonly used as a poison, generally externally. In this connection it is important to remember that its action on the skin is not quite as instantaneous as that of strong nitric acid, so that if water cannot be obtained immediately it is still possible to wipe off the acid with a dry rag

or cloth before the corrosion begins. The proper treatment is to wash it off or dilute it as much as possible with water, and then to neutralize any free acid with the milder alkalies such as bicarbonate of soda, magnesia, or even soap.

Uses.—Sulphuric acid is more widely used than any other product of chemical industry. It is one of the raw materials in the Leblanc soda process. It is used in making fertilizers, in the refining of petroleum, the manufacture of glucose, the preparation of nitric acid, ether, gun-cotton, nitroglycerin, and a number of other chemical substances, the extraction of indigo and other dye stuffs, the parting of gold and silver, and in numerous other industries. In medicine the acid is used but rarely, to promote digestion, and for other unimportant purposes; several of its salts, however, are of considerable value.

CARBONIC ACID.—H_2CO_3.

History.—Carbon dioxide, or carbonic acid gas, was the first gas to be distinguished from common air. It was observed by Van Helmont in the seventeenth century, who called it "gas sylvestre." It was very carefully studied by Black, in the middle of the last century, especially with regard to the change from caustic to mild or carbonated alkalies. Its composition was accurately determined by Lavoisier, some time after the discovery of oxygen.

Occurrence.—It is found in small quantities always present in the atmosphere, and especially so around volcanoes or over mineral springs. It invariably occurs where organic matters are being oxidized, whether by combustion, or by consumption in the body, or by the slower processes of decomposition and decay. Combined, it is found in enormous quantities in the form of the carbonates of calcium, magnesium, iron, zinc, sodium, and other metals, both as minerals or dissolved in water.

Preparation.—The simplest way to produce this gas is to decompose one of the carbonates, usually carbonate of lime, with some acid. The reaction is simply

$$CaCO_3 + H_2SO_4 = CO_2 + CaSO_4 + H_2O.$$

On a large scale, however, it is often more cheaply prepared by

igniting limestone, as in Lesson XIV., or by the combustion of coke or charcoal.

Properties.—(*a*) *Physical.*—Carbon dioxide is a colorless gas with a rather sharp taste and an acid smell. Its specific gravity is 1.529 compared to air as 1; and hence it can be caught in an open vessel by passing the gas in at the bottom and letting it rise and displace the air above. It dissolves readily in both water and alcohol, one volume of the former at 15° C. absorbing just about one volume of the gas. It supports neither combustion nor life. It can be liquefied without much difficulty, and is sometimes prepared for market in a liquid form.

(*b*) *Chemical.*—The dry gas possesses no acid properties, but when it dissolves in water a true acid, carbonic acid, is formed, which colors litmus paper red, and forms salts with metals and bases. With this acid, as well as with sulphuric and other diatomic acids, we have two distinct series of salts, the normal salts where the metallic atoms replace the hydrogen completely, and the acid or bi-salts, where half the hydrogen only has been replaced by the metal. The normal carbonates, excepting those of the alkaline metals, are insoluble in water and alkaline liquids, and hence serve as good tests for carbonic acid. The acid salts, on the other hand, are usually soluble, and, as they are formed by the action of CO_2 and water upon the normal carbonates, they furnish a simple way of dissolving the latter. Thus, in the case of lime,

$$\underset{\text{Calcic Hydrate.}}{CO_2 + Ca(OH)_2} = \underset{\text{Calcic Carbonate.}}{CaCO_3 + H_2O}$$

$$CaCO_3 + H_2O + CO_2 = \underset{\text{Calcic Bicarbonate.}}{Ca(HCO_3)_2}$$

$$Ca(HCO_3)_2 \text{ heated} = CaCO_3 + H_2O + CO_2$$

All these carbonates are readily decomposed by even quite dilute acids, with liberation of carbon dioxide and water.

(*c*) *Physiological.*—This gas is excreted from the body in large quantities, being produced by the oxidation of the carbon in the food. It also is the source of carbonaceous material to green plants. When inhaled in large quantities it exerts a peculiar anæsthetic effect, but it can hardly be considered a true poison, deaths from it resulting from suffocation. When present in unusu-

ally large amounts in the atmosphere, it is usually associated with a diminution in the quantity of oxygen present, and with the presence of other really deleterious bodies, carbonic oxide, poisonous nitrogenous bodies, and the like. When taken into the system in the form of effervescing beverages, it acts as a slight stimulant to the appetite and to digestion.

Uses.—Besides its functions in nature, this gas is prepared on a large scale and in a very pure form for the manufacture of carbonated waters. It is sometimes used for extinguishing fires, and the liquefied gas has been prepared for producing artificial cold. It is an important reagent, not only in the laboratory, but also in some quite important chemical industries, such as the manufacture of bicarbonate of soda and of potash, and other products.

NITRIC ACID.—HNO_3.

History.—This acid was, under the name of aqua fortis, one of the most valuable reagents known to the alchemists. Cavendish, 1784, determined its composition, producing it from nitrogen and oxygen in the presence of water.

Occurrence.—It is found in minute quantities free in the atmosphere, being produced by electricity. Its salts, especially the nitrates of soda, potash, and lime, are almost universally distributed wherever nitrogenous organic matter has undergone decomposition in the presence of mineral matters. Hence they are found, in small amounts, in all waters and in all soils; and in certain hot countries, where oxidation is rapid and the rainfall very intermittent, they can be extracted from the surface of the ground in large quantities. Potassic nitrate, or saltpetre, comes chiefly from India; while the sodic nitrate, or Chili saltpetre, comes from the deserts of Chili and Peru.

Properties.—(*a*) *Physical.*—Pure nitric acid is a colorless liquid, fuming in the air, of an acid odor and a very bitter and acid taste. It absorbs moisture quite readily, and when mixed with water it contracts slightly and rises in temperature, but not nearly so much as sulphuric acid.

(*b*) *Chemical.*—It is a powerful oxidizing agent, giving up its oxygen readily and itself decomposing according to the following reaction:

$$2HNO_3 = 3O + H_2O + 2NO.$$

The NO, which is a colorless gas, takes up oxygen from the air and is converted into the red gas NO_2.

In this way nitric acid oxidizes most organic substances, not only coloring matters, but tissues and fabrics of all sort. Upon the body it is extremely corrosive, acting instantaneously, and leaving deep yellow or orange stains, owing to the xantho-proteic reaction previously described, upon all proteid or albuminoid material—skin, nails, flesh, wool, or silk—with which it comes in contact.

Nitric acid acts readily upon the metals, oxidizing them according to the above reaction, and then in most cases dissolving the oxides thus formed to nitrates, with the production of water. Thus when copper is dissolved in nitric acid, the following reactions take place:

$$3Cu + 2HNO_3 = 3CuO + H_2O + 2NO,$$

and then $$3CuO + 6HNO_3 = 3Cu(NO_3)_2 + 3H_2O.$$

The cupric nitrate thus formed, like the cupric sulphate, is converted by caustic alkali into cupric hydrate, $Cu(OH)_2$, which dissolves in an excess of ammonia, in solutions of glucose, and, as in Fehling's solution, of Rochelle salt. Metallic iron is able to replace the copper in this and other acid solutions, depositing metallic copper and itself going into solution.

The only tests for this acid depend upon certain color reactions with various mineral and organic substances. With ferrous sulphate, in the presence of strong sulphuric acid, a peculiar dark brown liquid is produced, a compound of NO with two molecules of $FeSO_4$. This is an unstable compound and is readily decomposed by heat.

With organic bodies in general, the acid, especially in the presence of concentrated sulphuric acid, forms compounds containing the so-called radical nitryl, NO_2. Thus with phenol we form the compounds mono-, di-, and tri-nitrophenol (the latter is picric acid) according to circumstances, all of which bodies have a decidedly yellow or brown color. With anilin the color, which is not in all cases a very striking one, is produced by the formation of mono-, di-, and tri-nitroanilin.

The test with brucine is interesting as an example of the color

reactions by which the various alkaloids are distinguished from each other.

(*c*) *Physiological.*—Nitric acid, when concentrated, is almost as corrosive as oil of vitriol, and acts with greater rapidity. It needs the same treatment.

Uses.—This acid is largely used in the manufacture of various nitro-compounds—gun-cotton, nitroglycerin, picric acid, and others—used principally for explosives, and also in the preparation of the coal-tar colors. Several of the salts made from it, such as silver and lead nitrate, are important. Either this acid or its salts are essential to the manufacture of sulphuric acid, and the saltpetres form the most expensive constituent of gunpowder. The acid is occasionally employed in medicine as a caustic, and is a most important laboratory reagent, being constantly employed for reactions and tests.

LABORATORY EXPERIMENTS.

SULPHURIC, CARBONIC, AND NITRIC ACID.

I. **Sulphuric Acid.**—H_2SO_4.

1st. *Action on Organic Substances.*—Pour some common H_2SO_4 into a small beaker, and near it place a saucepan almost full of water. Dip a piece of filter paper in the acid, take it out *instantly*, and wash it well in the water. Notice that the filter paper has been converted into *parchment paper*, having a smooth, gelatinous texture, and greatly increased strength. Notice that a longer exposure destroys the paper. Also notice the rapid charring action of the common acid on paper, cloth, and pieces of wood, especially if they have been moistened first with water.

2d. *Action on Water.*—Half fill a small beaker with water, and into it pour slowly and carefully some of the common H_2SO_4. Notice how great a heat is generated by the mixture.

2d. *Tests for H_2SO_4 and for Soluble Sulphates:*

(*a*) *With Barium.*—Put some baric chloride, $BaCl_2$, in a test-tube and add a drop or two of diluted H_2SO_4 = white ppt. of $BaSO_4$. Notice that this ppt. is insoluble in acids

or in alkalies. Repeat this test, using magnesic sulphate, $MgSO_4$, instead of the H_2SO_4, and then notice how clearly the test shows, even with extremely dilute solutions of acid, or of $MgSO_4$, or of the $BaCl_2$ itself.

(*b*) *With Calcium.*—Put some calcic chloride, $CaCl_2$, in a test-tube and add a drop or two of diluted H_2SO_4 = white ppt. of $CaSO_4$. Repeat this test with more and more diluted acid, and when the limit of the test has been reached notice that the addition of $BaCl_2$ will still cause a ppt.

(*c*) *With Lead.*—Put some $Pb(C_2H_3O_2)_2$ in a test-tube, and to it add a few drops of diluted H_2SO_4 = white ppt. of $PbSO_4$. Notice how much more delicate this test is than the test for Cl with Pb salt, as under Section IV. in Lesson XI.

II. **Carbonic Acid.**—H_2CO_3 ($CO_2 + H_2O$).

Place limestone in a flask, add HCl dil., and pass the gas into water in a beaker, and also into a dry beaker. Smell and taste the water solution, and notice acid reaction of the solution to test paper, and also, if the paper be wet, of the gas. Dry the test papers and notice that the blue color returns.

Also,

(*a*) Light a match and plunge it into beaker of the gas; it is extinguished.

(*b*) Pass the gas into $Ca(OH)_2$ = ppt. of $CaCO_3$, which dissolves in an excess of the gas, and is precipitated from this solution by boiling.

Add some Na_2CO_3 to the following reagents in different test-tubes: $BaCl_2$, $MgSO_4$, $HgCl_2$, $Pb(C_2H_3O_2)_2$, and $AgNO_3$. Notice that in each case a ppt. is produced. Let them stand a little, pour away the top liquid, and to each add some HNO_3. Notice that in each case the ppt. dissolves, with evolution of CO_2.

To Na_2CO_3 in different test-tubes add a few drops of each of the following acids: HCl, HNO_3, H_2SO_4, $HC_2H_3O_2$. Notice that in each case CO_2 is liberated, even if the acid is much diluted.

III. **Nitric Acid.**—HNO_3.

(*a*) *Preparation.*—Place the saltpetre, K or $NaNO_3$, in a flask, cover it well with common H_2SO_4, coat the cork of the distilling-tube with paraffin, and carefully distil off the HNO_3 into a dry flask or beaker, as in Lesson V.

Do not clog the tube with paraffin; fit the cork tightly; wire in the cork if it keeps slipping out of the flask; heat gently, but keep the mixture boiling after the reaction once begins; let the contents of the flask cool before cleansing it, and avoid inhaling the vapors which pass over with the acid. If the vapors are very dense, hang near the apparatus some filter paper kept moistened with NH_4OH.

(*b*) *Tests.*—1st. *Action on Organic Substances.*—Add some of the distilled acid to indigo, fuchsin, and to test papers; notice that it is extremely acid and corrosive, and that in each case it alters and destroys the colors.

2d. *Action on Metals.*—Put a few drops of strong HNO_3 on a copper tack in a test-tube, and warm = reddish brown fumes, NO_2, and the metal dissolves. Dilute the solution, $Cu(NO_3)_2$, divide it into three parts, and test as follows:

(*a*) Add KOH = blue ppt. of $Cu(OH)_2$.

(*b*) Add NH_4OH carefully = same blue ppt., which dissolves in an excess of NH_4OH, forming a deep blue solution.

(*c*) Put in that solution an iron nail. Notice that the copper at once deposits on the nail, and that the iron dissolves, changing the color of the solution from blue to yellow. After a few minutes add NH_4OH to the solution = brownish red ppt. of $Fe_2(OH)_6$.

3d. *Ferrous Sulphate Test.*—Put in a test-tube half an inch of common H_2SO_4. Add gently a concentrated solution of $FeSO_4$, cool it, and down the sides of the test-tube run two or three drops of diluted HNO_3 = brown or black ring. Shaking or heating the liquids will spoil the test.

4th. *Phenol Test.*—Put in a test-tube one or two drops of phenol and about four times as much common H_2SO_4. Mix till they dissolve, and to the solution add a drop or two of much diluted HNO_3 = deep reddish-brown color.

5th. *Aniline Test.*—Put one or two drops of aniline in a small test-tube, full of H_2SO_4 dil. Mix till they dissolve. Place one or two drops of the solution in an evaporating-



dish, with several drops of common H_2SO_4, and stir with a rod dipped in much diluted HNO_3. Notice the red streaks, deepening to a dark red or brown color.

6th. *Brucine Test.*—Put in an evaporating-dish a few crystals of brucine, and dissolve them in a drop of common H_2SO_4. Stir with a rod dipped in much diluted HNO_3, and notice the deep red color, soon fading to a reddish yellow. Then add a drop of stannous chloride, $SnCl_2$, diluted three or four times with water, and the color changes to a reddish violet.

LESSON XIII.

PHOSPHORIC ACID, IRON, AND ALUMINIUM.

(ORTHO-) PHOSPHORIC ACID.—H_3PO_4.

Occurrence.—This acid never occurs free, but its salts are very widely distributed in nature. In the mineral kingdom they occur in many ordinary rocks, and invariably in the soil, while large and valuable deposits of phosphates of lime occur in many localities. Traces of phosphates are also found in most natural terrestrial waters. These salts occur in all the juices and hence the tissues of both plants and animals, and in almost all the latter they form by far the greater amount of the mineral matter of the skeleton.

Preparation.—The pure acid can be prepared by oxidizing phosphorus with nitric acid; on a large scale, however, it is usually obtained from bone ashes by treatment with acids, separation as calcium, lead, or barium phosphate, and decomposition of the latter by sulphuric acid.

Properties.—Phosphoric acid, when perfectly pure, can be obtained in the form of white crystals, very soluble in water. It is usually met with in the form of an aqueous solution, with no odor and, when not too strong, a pleasant acid taste. It forms with metals three series of salts, according to whether one, two, or three atoms of H are replaced. Thus, with sodium we have the normal sodic phosphate, Na_3PO_4, which is decidedly alkaline; the hydro-disodic phosphate, which is slightly alkaline; and the dihydro-sodic phosphate, or acid phosphate of soda, which has a decidedly acid reaction.

Tests.—The most delicate test for phosphoric acid and the soluble phosphates is the formation of a yellow crystalline compound with a nitric acid solution of ammonium molybdate. This precipitate, called commonly ammonio-phospho-molybdate, appears

with the least traces of phosphoric acid, and, while readily dissolved in alkalies, is very insoluble in acids, especially nitric acid. It contains only about 3% of phosphorus.

The ammonium magnesium phosphate, commonly, though erroneously, called "triple phosphate," is of more importance as a test for magnesium, and on account of its presence in urine, than as a test for this acid. It is discussed in the next lesson.

The argentic phosphate is of importance as distinguishing a solution of the ortho-salt or acid from solutions containing the meta- or pyro-phosphoric acid. The latter also form precipitates with argentic nitrate, but they are white.

IRON.—Fe.

Atomic weight, 56.

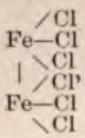
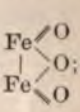
Occurrence.—This element is occasionally found in a free state either in the form of meteorites, or in igneous rocks as a product of reduction. In combination it is present in large or small quantities in all the ordinary rocks, in the soil, and even in all terrestrial waters. It is found in both animals and plants, and is an essential and probably the active ingredient of both chlorophyll and hæmoglobin.

Preparation.—Iron is extracted from its ores, which are either oxides or carbonates, by reduction with coal. The cast iron thus produced contains considerable carbon, besides silicon, and usually traces of sulphur, phosphorus, and manganese. It is converted into wrought iron, which is a very pure form of the metal, by a process of oxidation, and this is converted into steel by combining it with the proper percentage of carbon.

Properties.—(*a*) *Physical.*—Wrought iron is a rather soft, malleable, infusible metal of a specific gravity of 7.8. It differs from almost all other metals by having the property of welding, and also of becoming magnetic.

(*b*) *Chemical.*—The various compounds of iron may be divided into two distinct classes, which, excepting for the fact that the one can be converted into the other, are as separate as if they belonged to two different elements. In the ferrous compounds one atom of iron is present, and is considered to have two "bonds,"

i.e., to be able to unite with two monatomic atoms or radicals like chlorine or hydroxyl, OH, or with one diatomic atom or radical, like oxygen or the SO_4 group. Thus, ferrous chloride is $FeCl_2$; ferrous hydrate $Fe(OH)_2$; ferrous nitrate $Fe(NO_3)_2$, etc.: while ferrous oxide is FeO, ferrous sulphate $FeSO_4$, and ferrous carbonate $FeCO_3$.

The ferric compounds, on the other hand, contain two atoms each of iron, combined together with one bond, and with three bonds from each atom, or six bonds for the two, to unite with other elements. Hence the graphic formula for ferric chloride, Fe_2Cl_6, would be  and for ferric oxide, Fe_2O_3, ; and the formula for ferric hydrate is $Fe_2(OH)_6$, and for ferric sulphate is $Fe_2(SO_4)_3$.

These compounds can be converted one into the other by the processes of either oxidation or reduction. Thus the precipitates of the ferrous salts will absorb oxygen even from the air, and be converted into ferric compounds—thus,

$$\underset{\text{Ferrous Hydrate.}}{2Fe(OH)_2} + O + H_2O = \underset{\text{Ferric Hydrate.}}{Fe_2(OH)_6}.$$

While the latter, if treated with hydrogen or SO_2 or some other powerful reducing agent, will lose oxygen and be converted into the corresponding ferrous salt. Where nitric acid is used for oxidizing, the oxygen is derived from it instead of from the atmosphere, and water and NO are liberated, as in the last lesson.

Tests.—Among the more important tests for both series of these iron compounds are those made by the ferro- and ferricyanides of potash. The latter may be considered as salts of ferro- and ferricyanic acids, H_4FeCy_6 and $H_6Fe_2Cy_{12}$, where the acid radicals are composed of a compound of iron, ferrous and ferric, with molecules of cyanogen or CN. The ferrous salt of ferricyanic acid, or $Fe_3Fe_2Cy_{12}$, is a well-known blue pigment, almost as important as the Prussian blue, the ferrocyanide of ferric iron, $Fe_4(FeCy_6)_3$. By means of these two tests it is easy to distinguish the presence of one or both varieties of iron in a solution.

The solution, however, must be slightly acid, for alkalies destroy the colors, and strong acids alter the reagents.

The tannate of iron, made by adding tannic acid to a neutral solution of ferric iron, has been known for many centuries as a test for iron, and until recently was the source of almost all the ink of commerce.

The best test for ferric iron is the formation of ferric sulphocyanide by the addition of ammonic or other soluble sulphocyanide to a neutral or slightly acid ferric solution. No precipitate is formed, but the compound, when dissolved in water, gives a very characteristic blood-red color, which shows even in very dilute solutions. This color is destroyed by reducing agents or by alkalies, but is restored by oxidation and by acids.

ALUMINIUM.—Al.

Atomic weight, 27.

History.—Salts of this metal, especially the alums, have been known since the Middle Ages, if not earlier. The metal itself was first isolated by Oersted in 1824, and Wöhler in 1827, and has been prepared on a commercial scale since about 1850.

Occurrence.—It is the most widely distributed metal in the earth's crust, never occurring free, but generally associated with other metals and combined with silica to form the great mass of common rocks. From their decomposition come the many varieties of clay, all more or less pure silicate of alumina. It is not absorbed, however, to any great extent by most plants, nor does it enter into the composition of animals.

Preparation.—The metal is now being extracted from its salts by electricity, but it has usually been obtained by heating them with metallic sodium. The sodium combines with the chlorine or other constituent of the salt, and liberates the aluminium. Improvements are being constantly made in the process, and great efforts are being made to cheapen the metal so as to bring it into general use.

Properties.—(*a*) *Physical.*—Aluminium has many very valuable properties. It has a bright white color, and its surface polishes well and does not readily tarnish. It is very strong, and can be

easily worked by hammering, drawing, and to some extent by casting. It is very light (specific gravity, 2.6), and is a good conductor of heat and electricity.

(*b*) *Chemical.*—Aluminium is not attacked by nitric acid, but dissolves readily in hydrochloric and sulphuric acids, forming salts similar to those of ferric iron. When heated with potassic or sodic hydrates, it dissolves with evolution of hydrogen and the formation of salts, aluminates, of the metals. Thus:

$$Al_2 + 2KOH + 2H_2O = K_2Al_2O_4 + 3H_2. \qquad \text{I.}$$

Potassic Aluminate.

This same compound is formed when aluminic hydrate, $Al_2(OH)_6$, is heated with caustic potash. Thus:

$$Al_2(OH)_6 + 2KOH = K_2Al_2O_4 + 4H_2O. \qquad \text{II.}$$

These aluminates are decomposed by an excess of ammonic chloride, and the Al is precipitated as aluminic hydrate. Thus:

$$K_2Al_2O_4 + 2NH_4Cl + 4H_2O = Al_2(OH)_6 + 2KCl + 2NH_4OH. \qquad \text{III.}$$

Alums.—Some important compounds, many of which contain this metal, are grouped together under the name of alums. These substances all have a striking resemblance to each other in crystalline form and in general composition, and in short in most of their physical and chemical properties.

The common or potash alum has the composition $K_2Al_2(SO_4)_4 + 24H_2O$, and is therefore a hydrated double sulphate of potassium and aluminium. The other alums differ from this in that other metals are substituted for the potassium and aluminium. Thus, instead of potassium we may have any of the alkaline metals, Na, NH_4, or Li; and for aluminium may be substituted iron or chromium, making the iron and chrome alums of commerce. Hence we have a whole series of different alums, such as—

Soda alum, $Na_2Al_2(SO_4)_4 + 24H_2O$.

Chrome alum, $K_2Cr_2(SO_4)_4 + 24H_2O$.

Ammonia iron alum, $(NH_4)_2Fe_2(SO_4)_4 + 24H_2O$.

On heating an aluminium alum before the blowpipe or otherwise, it melts at quite a low temperature (potash alum at 92° C.), and on further heating the water of crystallization gradually boils away, leaving a white porous mass known as burnt

alum. This mass, when strongly heated with the addition of a small amount of cobaltic nitrate, forms a compound of aluminium and cobalt known as Thénard's blue. This is prepared on a large scale and used as a paint.

Tests.—The test for this metal most generally employed is the precipitation of its hydrate by means of an alkaline hydrate. Thus:

$$K_2Al_2(SO_4)_4 + 6NH_4OH = Al_2(OH)_6 + 3(NH_4)_2SO_4 + K_2SO_4 \quad \text{IV.}$$

This precipitate is almost colorless, and, in case any ferric salts were present, would be entirely obscured by the accompanying brown ferric hydrate. The aluminic hydrate differs, however, from ferric hydrate, in that it dissolves, as we have seen in Equation II., on heating with KOH, and, as in Equation III., is re-precipitated by NH_4Cl. Hence, when it is desired to separate iron from aluminium in any solution, it is only necessary to add an excess of KOH and boil, when the Fe will precipitate as $Fe_2(OH)_6$ and can be filtered out, while the Al remaining in the filtrate can afterward be precipitated by NH_4Cl.

Lakes.—An important property of the Al salts is that of forming colored insoluble compounds called lakes, with most of the dye stuffs. This is very valuable in dyeing and calico printing, for it renders it possible to fasten the colors upon the cloth. The fabric can be first printed with or steeped in a solution of alum, and dried. Then, on passing it into an alkaline bath of the color, the lake will be precipitated wherever the alum was spread, forming a close flocculent compound which on drying adheres firmly around the individual fibres.

Uses.—The metal aluminium is used more and more every year, as its cost is diminished, for purposes where its lightness, strength, and good color are worth the extra expense. The salts, especially the alums, are largely used in dyeing, in tanning leathers, and in some other industries.

LABORATORY EXPERIMENTS.

PHOSPHORIC ACID, IRON (FERROUS AND FERRIC), AND ALUMINIUM.

I. **Phosphoric Acid.**—H_3PO_4.—Test some solution of hydro-di-sodic phosphate, Na_2HPO_4, as follows:

1st. Fill a test-tube nearly full of ammonic molybdate $(NH_4)_2MoO_4$, add a drop or two of HNO_3, and then a few drops of the solution = yellow crystalline ppt. Notice under the microscope the star-shaped crystals.

2d. To the solution in a test-tube add NH_4OH till it smells of ammonia, and then a little $MgSO_4$ = white crystalline ppt., $MgNH_4PO_4$. Notice under the microscope the white, feathery crystals.

3d. To the solution in a test-tube add a few drops of $AgNO_3$ = yellow ppt., Ag_3PO_4. This ppt. dissolves in HNO_3 as well as in NH_4OH.

II. **Ferrous Iron.**—FeO—Make a weak solution of $FeSO_4$ and test as follows:

1st. Add potassic ferrocyanide, K_4FeCy_6 = light blue ppt.

2d. Add potassic ferricyanide, $K_6Fe_2Cy_{12}$ = deep blue ppt.

Notice that both these ppts. are decomposed by alkalies.

3d. Add KOH = greenish ppt., $Fe(OH)_2$, turning brown slowly in the air.

4th. Add $(NH_4)_2CO_3$ = greenish-white ppt., quickly turning green.

III. **Ferric Iron.**—Fe_2O_3—To a solution of $FeSO_4$ add a few drops of H_2SO_4 dil., and boil. To the hot liquid add a few drops HNO_3 conc., and heat again. Repeat this until the liquid has a yellow or brown color. Then test this solution and also some diluted Fe_2Cl_6, as follows:

1st. Add K_4FeCy_6 = deep blue ppt., Prussian blue.

2d. Add $K_6Fe_2Cy_{12}$ = dark green solution. To this add a few drops of $SnCl_2$ = deep blue ppt.



Notice that both these ppts. are decomposed by alkalies.

3d. Add KOH = reddish brown ppt., $Fe_2(OH)_6$.

4th. Add $(NH_4)_2CO_3$ = reddish brown ppt., with evolution of CO_2.

5th. Add a solution of tannin in water = bluish black ppt. (ink).

Notice that this dissolves in acid; hence, if solution is very acid, must nearly neutralize with NH_4OH before adding the tannin.

6th. Add ammonic sulphocyanide, NH_4CNS = deep red color, which is destroyed by alkalies. Compare the delicacy of Tests 1st, 5th, and 6th by tests on very dilute solutions.

Dissolve some iron nails in HCl conc., nearly neutralize with NH_4OH, and test for ferrous and ferric salts with K_4FeCy_6 and with $K_6Fe_2Cy_{12}$. Also test for ferric salts with tannin and with NH_4CNS.

IV. **Aluminium.**—Al.—(*a*) Place piece of Al foil in a test-tube with one inch of KOH, and warm. The Al dissolves with escape of H_2 gas. To solution add ammonic chloride, NH_4Cl in excess = ppt. of $Al_2(OH)_6$.

(*b*) Place a little dry alum on charcoal and heat in a blowpipe flame; it boils and becomes burnt alum. To this add a drop of cobaltic nitrate, $Co(NO_3)_2$, and heat again = a blue mass.

(*c*) Make concentrated solutions both of aluminic sulphate, $Al_2(SO_4)_3$, and of potassic sulphate, K_2SO_4, by treating them in separate test tubes with a little hot water. Mix together in a test tube equal quantities of these two solutions, and notice the resulting precipitate of potash alum, $K_2Al_2(SO_4)_4 + 24H_2O$. Examine this ppt. under the microscope.

Dissolve the dry alum in a little hot water, put a drop of the solution on a slide, let it stand quietly for several minutes till it has partly dried, and then examine, under the low power of the microscope, the crystals of alum.

Dilute the rest of the solution and test as follows:

1st. Add NH_4OH = ppt. of $Al_2(OH)_6$, insoluble in excess of NH_4OH.

2d. Add KOH = same ppt., which dissolves in excess of KOH. To this solution add NH_4Cl in excess = ppt. of $Al_2(OH)_6$.

3d. Mix a few drops of Fe_2Cl_6 with the alum solution, and sep-

arate the Fe and Al as follows. To mixed solution add KOH in excess and boil = ppt. of $Fe_2(OH)_6$. Filter, and to filtrate add excess of NH_4Cl = ppt. of $Al_2(OH)_6$.

4th. Mix with the alum solution some solution of cochineal, add NH_4OH, and boil. Notice the red ppt. (crimson lake).

5th. Mix with the alum solution some extract of litmus, add NH_4OH, and boil. Notice the blue ppt. (lake). Filter off these ppts. and notice the lack of color in the filtrates.



FIG. 1. Ammonio-Phospho-Molybdate, x 150.



FIG. 2. Triple Phosphate, x 250.



FIG. 3. Alum Crystals (large), x 30.



FIG. 4. Alum Crystals (small), x 150.



FIG. 5. Calcium Sulphate, x 150.

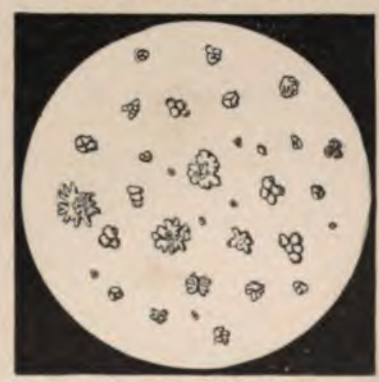


FIG. 6. Calcium Carbonate, x 200.

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LESSON XIV.

CALCIUM AND MAGNESIUM.

CALCIUM.—Ca.

Atomic weight, 40.

History.—The metal was first prepared by Davy in 1808. Its compounds, however, had been known and used for mortar and cements since the earliest ages.

Occurrence.—This metal is never found free, but in combination is, next to aluminium, the most abundant metal in the earth's crust. It occurs as carbonate under the forms of limestone, marble, chalk, all of which are originally formed from the shells of marine animals and coral. It is found as a silicate associated with Al, Fe, Mg, and other metals in the common rocks and in the soil, and the sulphate, fluoride, phosphate, and other compounds are among the well-known minerals. As sulphate or bicarbonate it occurs in all terrestrial waters, and as phosphate and to some extent as carbonate it is present in the juices and tissues of both plants and animals and especially in bones of vertebrates.

Preparation.—The metal calcium can be prepared from its salts by electricity or by reduction with metallic sodium. Its salts are all derived from limestone either by the action of heat or of acids.

Properties.—(*a*) *Physical.*—The metal calcium is not important, being expensive to prepare, and oxidizing readily in moist air. It has a yellow color, is tenacious and malleable, is a little harder than lead, and has a specific gravity of only 1.6. It burns in the air when heated to redness, and it decomposes water, with evolution of hydrogen.

(*b*) *Chemical.*—Of its different compounds the carbonate is the most important. This compound, when heated to a dull red

heat, gives off CO_2 and changes to lime, CaO, according to the simple formula,

$$CaCO_3 \text{ heated} = CaO + CO_2.$$

This is done on a large scale in lime kilns, and the resulting product is the quicklime of commerce. The latter occurs in white, earthy looking lumps, which must be kept from the atmosphere or they will "air-slake," *i.e.*, absorb CO_2 from the atmosphere and re-form the original $CaCO_3$. When water is added to them they slake, *i.e.*, become converted, with change of form and evolution of heat, into slaked lime, or calcic hydrate, $Ca(OH)_2$.

This is a white powder, slightly soluble in water, forming a weak alkaline solution known as lime water. Mortar is made by mixing fresh slaked lime with sharp clean sand, and when it sets it absorbs CO_2 from the atmosphere and changes back to calcium carbonate, setting free water. Thus,

$$Ca(OH)_2 + CO_2 = CaCO_3 + H_2O.$$

The same reaction takes place when limewater is used as a test for carbon dioxide.

Calcium carbonate, precipitated from calcium solutions by CO_2 or by soluble carbonates, is a white crystalline substance, insoluble in water and alkaline solutions, but readily dissolved by acid, even if much diluted. It also dissolves, as before mentioned, in CO_2 and H_2O, forming the soluble bicarbonate $CaH_2(CO_3)_2$.

Tests.—The formation of calcium sulphate, by the addition of sulphuric acid or a soluble sulphate, is not a very delicate test for lime, for it dissolves to a slight extent in water as well as in acid solutions. It can, however, be prepared without trouble in all concentrated solutions, and it forms characteristic well-defined, white, needle-shaped, radiating crystals.

The best test, however, for lime salts, and the one most commonly used, is the formation of oxalate of lime by the addition of solutions of oxalic acid or of alkaline oxalates. Magnesium salts are not precipitated with these reagents, so this presents an easy method for separating the two metals. The calcic oxalate thus formed is a white powder, insoluble in neutral and alkaline solutions, and also in weak acids, though dissolving readily in strong

acids. When precipitated quickly from concentrated solutions, it has no characteristic shape; but when allowed to form very slowly from dilute solutions, it sometimes appears in granular or dumb-bell forms; but more usually, mixed with irregular little plates and crystals, it crystallizes in some of the characteristic octahedra which we meet with in urine.

MAGNESIUM.—Mg.

Atomic weight, 24.3.

History.—The salts of magnesium were recognized at an early date, but the metal was not isolated until 1808, by Davy.

Occurrence.—Magnesium always occurs in combination. It is found in the mineral kingdom, usually associated with calcium and other metals, as carbonate, phosphate, chloride, and especially as silicate. It occurs in most waters, especially sea and mineral waters, as chloride, bicarbonate, and sulphate, and it is found in both plants and animals, usually as a phosphate.

Preparation.—The metal is prepared in considerable quantities either by electrolysis or by the reduction of the chloride with metallic sodium.

Properties.—(*a*) *Physical.*—Magnesium is a silver-white, lustrous metal, which tarnishes quite readily in moist air. It is very light, (specific gravity = 1.7) and is malleable and ductile.

(*b*) *Chemical.*—On heating in the air and especially in pure oxygen, magnesium burns to magnesia (MgO) with a brilliant white flame, which is very rich in actinic rays. It is unaffected by dilute alkalies, but dissolves readily in dilute acids with evolution of hydrogen.

The magnesia obtained in this way (magnesia usta) is prepared on a large scale by burning magnesium carbonate in crucibles or retorts. It is a white powder, dissolving slightly in cold and hardly at all in hot water, to a faint alkaline solution of magnesic hydrate, $Mg(OH)_2$. It is somewhat used in medicine as a mild antacid. The magnesic hydrate is also produced by the action of alkaline hydrates upon solutions of magnesium. This compound dissolves readily in acids and also in solutions of ammonium salts, especially of ammonium chloride. Hence when-

ever we wish to make alkaline a solution containing magnesium, and yet do not wish to precipitate that metal, we always take pains to add beforehand a certain amount of NH_4Cl.

The best test by far for magnesium compounds is the formation of the ammonio-magnesium phosphate or triple phosphate, $MgNH_4PO_4$, by the addition of ammonic hydrate and hydrodisodic phosphate. This precipitate comes down readily in the form of characteristic white, feathery or fern-leaf crystals, which contain six molecules of water of crystallization, and are somewhat soluble in water, especially if warm, but much less soluble in dilute ammonic hydrate. They dissolve readily in acids. When precipitated very slowly they gradually tend to form the rectangular, rhombic, coffin-lid crystals which are met with in decomposing urines.

LABORATORY EXPERIMENTS.

CALCIUM AND MAGNESIUM.

I. **Calcium.**—Ca.—Heat a piece of limestone on charcoal with blowpipe = CaO. Place it in a small evaporating-dish with a little water = $Ca(OH)_2$. Test this liquid as follows:

1st. Notice that it is slightly alkaline to litmus and to turmeric papers.

2d. Add NH_4OH and ammonic oxalate, $(NH_4)_2C_2O_4$, and warm = ppt.

Place a lump of quicklime in a dish, moisten it with water and let it stand = slaked lime. Add some more water and test the filtered solution, $Ca(OH)_2$, as follows:

1st. With test papers = alkaline.

2d. With $(NH_4)_2CO_3$ or with Na_2CO_3 = white ppt., $CaCO_3$.

3d. Blow into some of the solution with a pipette = same white ppt. Let some stand awhile, and then examine the crystals of $CaCO_3$ under the microscope.

4th. Add a drop of NH_4OH and then $(NH_4)_2C_2O_4$ = white ppt. of calcic oxalate, CaC_2O_4. Notice that this ppt. dissolves in strong acids and is reprecipitated by alkalies. In a test-tube put some very dilute solution of $CaSO_4$, and a drop or two of diluted $(NH_4)_2C_2O_4$,



let it stand as long as possible, and at the end of the lesson examine under the microscope for crystals of CaC_2O_4.

Put some $CaCl_2$ in three test-tubes and to these add respectively some H_2SO_4 dil., some $(NH_4)_2SO_4$ and some $MgSO_4$. In each there will be a heavy white ppt. of $CaSO_4$. Examine the crystals under the microscope.

II. **Magnesium.**—Mg.—Burn in the forceps a piece of Mg tape = MgO. Place this in a dish with a few drops of water, and notice that the liquid is slightly alkaline to test papers. Add some HCl dil. = solution of $MgCl_2$. Test this solution, and also some $MgSO_4$, diluted, as follows:

1st. Add NH_4OH to the $MgCl_2$ solution = no ppt.; then add Na_2HPO_4 = white ppt., $MgNH_4PO_4$, the so-called *triple phosphate.*

2d. Add NH_4OH to the $MgSO_4$ solution = ppt. of $Mg(OH)_2$. To this add NH_4Cl; notice that the ppt. redissolves. Then add Na_2HPO_4 = same white ppt. of $MgNH_4PO_4$. Notice that this ppt. dissolves in acids and is reprecipitated by alkalies. Examine it under the microscope = feathery crystals.

Make this ppt. very slowly with much diluted solutions of $MgSO_4$ and of Na_2HPO_4, and notice variations in the crystals thus formed.

III. **Separation of Calcium and Magnesium.**—Mix some $MgSO_4$ with three or four times as much $CaSO_4$. To one inch of the mixture add half an inch of NH_4Cl and a few drops of NH_4OH. Then add half an inch of $(NH_4)_2C_2O_4$ and warm = ppt. of CaC_2O_4. Filter off this, to filtrate add half an inch of Na_2HPO_4, and let stand = ppt. of $MgNH_4PO_4$.

IV. **Separation of Iron, Calcium, and Magnesium.**—Mix a little Fe_2Cl_6 with the above mixture of $CaSO_4$ and $MgSO_4$. To this, in a small beaker, add NH_4Cl and some NH_4OH = ppt. of $Fe_2(OH)_6$. Filter off this, dissolve it in HCl, and test this solution for ferric iron with NH_4CNS and with $K_4Fe_2Cy_6$. To the filtrate add $(NH_4)_2C_2O_4$ and warm = ppt. of CaC_2O_4. Filter and to filtrate add Na_2HPO_4 = ppt. of $MgNH_4PO_4$.

Repeat the above separation of Fe, Ca, and Mg on a piece of common "dolomitic" limestone, *i.e.*, limestone containing Mg and frequently Fe, dissolving it in a little HCl dil., and treating the solution according to the above directions.

LESSON XV.

THE ALKALINE METALS (AMMONIUM, SODIUM, POTASSIUM, AND LITHIUM), WOOD ASHES, ACIDIMETRY AND ALKALIMETRY.

THE ALKALINE METALS.

The metals of this group are characterized by the alkaline properties of their carbonates and some of their phosphates, as well as of their oxides and hydrates. These alkaline properties are shared by the oxides and hydrates of the alkaline earthy metals, Ca, Mg, Ba, and Sr, but the carbonates and phosphates of the latter are always neutral. The more prominent members of the group are the metals sodium and potassium and the compound radical ammonium; lithium, cæsium, and rubidium also belong to the group, but are of less importance.

AMMONIUM.—NH_4.

This name is given to a compound basic radical, formed from nitrogen and hydrogen, which is able to take the place of monatomic metals in all compounds, and which in almost every respect behaves like a true metal of the alkaline group.

Tests.—Its compounds are readily distinguished by being easily decomposed into ammonia, NH_3. This gas is freely evolved from ammonic hydrate, and is set free from more stable ammonium compounds by heating them with caustic potash or soda, after the formula,

$$NH_4Cl + KOH = NH_3 + KCl + H_2O.$$

The NH_3 is recognized by its characteristic odor, by its temporary alkalinity to wet test papers, and by the formation of white fumes of NH_4Cl when brought near HCl vapors.

Almost all the ammonium compounds, as well as those of the other alkaline metals, are readily soluble in water. With platinic

chloride, however, it forms a yellow crystalline precipitate, which is slightly soluble in water and less so in diluted alcohol. The crystals are a characteristic form of the isometric class, often occurring in regular octahedra. The crystals are very similar to the similar potassium compound, but if ignited are converted into spongy platinum, while the potassium salt changes into metallic platinum and potassium chloride.

SODIUM.—Na.

Atomic weight, 23.

History.—This metal was prepared by Davy in 1807, and has since been produced on a large scale for the manufacture of aluminium, magnesium, and other metals.

Occurrence.—It never occurs free, but is found universally distributed, chiefly as chloride, but also as phosphate, silicate, carbonate, sulphate, etc., in minerals, the soil, water, air, and all the fluids and tissues of plants and animals.

Preparation.—The metal is often prepared by electrolysis, but is more usually extracted from NaOH or Na_2CO_3 by reduction with carbon or iron.

Properties.—Sodium is a very light metal, of bright metallic lustre when freshly cut, but tarnishing readily whenever exposed in the least to either air or moisture. When thrown on water it floats, at the same time decomposing the water and setting free hydrogen with such an evolution of heat that it usually ignites. The combustion in most cases is terminated by a little explosion. The formula is

$$Na_2 + 2H_2O = 2NaOH + H_2.$$

The sodic hydrate, produced by this reaction, is an amorphous, very hygroscopic, solid substance, which dissolves very readily in water, making a strongly alkaline, caustic solution.

Tests.—The most delicate test that we possess for sodium is indeed the most delicate test we possess for any substance, and is almost useless for that reason. It is the production of the yellow flame when sodium vapor is heated to incandescence. In the spectroscope this flame is resolved into two very closely adjacent lines lying between the yellow and orange and corresponding to Fraunhofer's line D in the solar spectrum. This test, however, is so

delicate that it will show the presence of sodium in any substance exposed to dust.

A test which is sometimes employed, and which is interesting as showing almost the only insoluble Na salt we have, is the formation of sodium antimonate by adding to a sodium solution a freshly prepared neutral solution of potassium antimonate, $KSbO_3$. The crystals in this case are sometimes octahedra, not unlike in shape some of the potassic and ammonic platinic chloride crystals. Generally, however, the crystals have a rectangular form, as seen in Fig. 5, Plate III.

The crystals of NaCl are much larger than any of the rest in this lesson, and, though of the isometric class, are generally in the form of cubes, not of octahedra.

POTASSIUM.—K.

Atomic weight, 39.11.

This element was separated at the same time and is prepared in much the same way as the metal sodium. Greater care, however, is necessary in its manufacture, for it is liable to form explosive compounds when it is reduced.

Properties and Tests.—Its properties are much the same as those of sodium, its color being more bluish-white than the other, and its vapor, when it burns, having a distinctly violet hue. This cannot be seen in the presence of the strong yellow flame of sodium unless the latter is observed by the interposition of a plate of cobalt blue glass. It is always advisable, however, when testing for potassium by the flame test, to compare it with the flame from a known potassium compound, for the light that passes through the blue glass from a strong sodium flame is itself not so very far from violet.

The compound with platinic chloride is very similar in appearance to that formed by ammonium salts.

LITHIUM.—Li.

Atomic weight, 7.

This metal has itself no practical value, but its salts, especially the carbonate and citrate, have achieved some importance as a remedy for gout, rheumatism, and kidney diseases.

There is an excellent qualitative test for it in the carmine red flame when its salts are volatilized in the Bunsen flame. When examined in the spectroscope, this appears as a deep and characteristic red line.

The metal is usually separated from the accompanying sodium and potassium compounds by precipitation with hydro-disodic phosphate.

Wood Ashes.—The ashes of a plant represent the mineral matter that its roots have been able to extract from the soil. Almost all the rest of the plant has been built up from CO_2 and water by means of chlorophyll. The percentage of this ash varies from 2 or 3% in the case of some of the common hard woods to 20 or 25% in the case of tobacco. A large variety of metals and acids are found in them, most of which seem essential to one or another function of the plant. Thus, if the soil does not contain iron, the leaves will be pale and the plant will lack nourishment from loss of chlorophyll. If phosphorus be not present, the seeds will not mature. If, in some plants, silica be absent, the stems will lack stiffness. The main bulk of the ordinary ash is composed of potassium carbonate, the roots of land plants preferring to extract that metal, while those of sea plants show a preference for sodium. The tests for the various ingredients simply apply the reactions already described.

Acidimetry and Alkalimetry.

The principles on which the determination of the percentage of acid or alkali in a solution is based are exceedingly simple.

We possess certain coloring bodies, which have the property of changing color when the reaction of their solution is changed. Of these, phenol-phthaleïn and orange No. 2 are at present the most used, although litmus, cochineal, Congo red, and many others are available for the same purpose. These substances can be used as indicators, to show by their change of color when enough of a standard acid or alkali solution has been added to exactly neutralize the solution.

The standard solutions, in this lesson, are all "normal;" *i.e.*, they are of such a strength that one cubic centimetre of solution contains the hydrogen equivalent of the substance, weighed in milligrammes. Accordingly all normal solutions correspond to

each other in strength, one c.c. of one solution being exactly equivalent to one c.c. of any other.

Now, potassic hydrate, KOH, like HCl, HNO_3, and other substances, is univalent, *i.e.*, equivalent to one atom of hydrogen. Hence 1 c.c. of the normal alkali solution contains 56 milligrammes (K = 39, O = 16, H = 1; 39 + 16 + 1 = 56), or 0.056 gramme of pure KOH.

Sulphuric acid, however, H_2SO_4, which is used for the standard acid solution, is divalent, *i.e.*, equivalent to two atoms of hydrogen. Hence 1 c.c. of its normal solution contains only 49 milligrammes, corresponding to half its molecular weight, of pure H_2SO_4.

The normal solutions of other divalent substances, like oxalic acid, $H_2C_2O_4$, and sodic carbonate, Na_2CO_3, also contain, in one c.c., a number of milligrammes of the substance equal to one-half the molecular weight.

Hence, by comparing the combining power of a substance with its molecular weight, we can readily determine the equivalence factor of any normal solution.

LABORATORY EXPERIMENTS.

THE ALKALINE METALS—WOOD ASHES—ACIDIMETRY AND ALKALIMETRY.

I. **Ammonium, NH_4.**—Put some NH_4OH in a beaker and test.

1st. Wet some litmus and turmeric papers and hold them in the fumes. The color changes, but returns on drying.

2d. Dip a clean glass rod in HCl conc., and place it over the beaker = white fumes of NH_4Cl. Repeat this test with HNO_3 conc.

Place in a beaker some NH_4Cl; add KOH and heat. Smell the fumes and test for NH_3 as in Tests 1st and 2d.

Put in test-tube a quarter of an inch of NH_4Cl, and add a few drops of $PtCl_4$ = yellow crystalline ppt. $(NH_4)_2PtCl_6$. Examine it under the microscope.

II. **Sodium, Na.**—Place a very small piece of sodium on wet

filter-paper in a saucepan one-third full of water. Test the solution as follows:

1st. Notice that it is permanently alkaline to test-papers.

2d. Dip the Pt wire in the liquid and heat it with a Bunsen burner; notice the yellow color of the flame.

Dissolve as much NaCl as possible in a test-tube with hot water.

3d. Put in a test-tube about a quarter of an inch of the solution, and to this add an equal quantity of a solution of potassium antimonate, $KSbO_3$. Let it stand for fifteen or twenty minutes and then examine the crystals of $NaSbO_3$ under the microscope.

4th. Put a drop of the hot solution on a slide, let it evaporate a little, and examine the cubical salt crystals under the microscope.

III. **Potassium, K.**—Place a very small piece of potassium on wet filter paper in a saucepan one-third full of water. Test the solution as follows:

1st. Notice that it is permanently alkaline to test papers.

2d. Dip the Pt wire in KOH and heat it with a burner; examine the flame through a piece of blue glass = violet. This test is shown better below with the wood ashes.

Put in a test-tube a quarter of an inch of KOH, fill it nearly full with equal quantities of alcohol and water, acidulate with HCl dil., and then a drop or two of $PtCl_4$ = yellow crystalline ppt., K_2PtCl_6. Examine the crystals under the microscope.

N.B.—To obtain good crystals of this and of the similar ammonium compound, it is best to ppt. slowly from dilute solutions.

IV. **Lithium, Li.**—Test Li_2CO_3 as follows: Place it in a small dish and add two or three drops of HCl dil., until it just dissolves. Dip the Pt wire in this and heat in the burner = deep red flame.

Put in a test-tube a quarter of an inch of Na_2HPO_4 and half an inch of NH_4OH; into this pour the LiCl solution and warm gently = white ppt., Li_2HPO_4.

V. **Wood Ashes.**—Mix one-half the ashes with water, filter, and test the solution.

1st. With test papers = alkaline.

2d. Add some HNO_3 conc., and notice the effervescence of CO_2; then add a drop or two of $AgNO_3$ = slight ppt. of $AgCl$.

To the rest of the ashes add HCl conc., and examine some of the mixture on the Pt wire in the flame for *Na* and *K* as above.

Then add water, filter, and test the solution in different test-tubes, as follows:

1st. Add $BaCl_2$ = ppt of $BaSO_4$.

2d. Add NH_4CNS = red color due to *Iron*.

3d. Add NH_4OH till alkaline; notice ppt. of $Ca_3(PO_4)$ and of $Fe_2(OH)_6$. Filter, and to filtrate add $(NH_4)_2C_2O_4$=ppt. of CaC_2O_4.

4th. Filter off the CaC_2O_4 in 3d, and to filtrate add Na_2HPO_4 and let stand. Notice white crystalline ppt. of $MgNH_4PO_4$.

VI. **Acidimetry and Alkalimetry.**—Place in a beaker five c.c., carefully measured with the pipette, of KOH, and a drop or two of the phenol-phthaleïn solution. To the mixture add with great care from the burette the normal standard acid solution until the red color just disappears. Read off the burette and calculate the quantity KOH as below. Repeat this test, using Orange No. 2, instead of phenol phthaleïn, as an indicator; in this case stop pouring in the acid directly the color changes from orange to pink.

Also test in the same way the strength of the Na_2CO_3 solution, using Orange No. 2 as an indicator. Try to make the test with phenol-phthaleïn instead, and notice how the evolution of CO_2 interferes with the indicator.

Make the same tests upon the H_2SO_4 dil. and the HCl dil., using the normal standard alkali solution instead of the acid solution, and trying in one case phenol-phthaleïn and in the other case Orange No. 2 as indicators.

To calculate the results remember that 1 c.c. of normal standard acid or alkali solution is equivalent to 0.049 gm. H_2SO_4, 0.0365 gm. HCl, 0.056 gm. KOH, and 0.053 gm. Na_2CO_3. We can call these decimals the equivalence factors of their respective compounds.

Then the quantity of one of these compounds in the solution tested is equal to the corresponding equivalence factor, multiplied by the number of c.c. of standard normal solution used.



FIG. 1. Triple Phosphate, x 250.
(Rapid precipitation.)



FIG. 2. Triple Phosphate, x 250.
(Same as Fig. 1, after standing 12 hours.)



FIG. 3. Calcium Oxalate, x 350.



FIG. 4. Sodium Chloride, x 50.



FIG. 5. Sodium Antimonate, x 300.



FIG. 6. Ammonium (or Potassium) Platinic Chloride, x 150.

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PART V.

WATER ANALYSIS.



LESSON XVI.

WATER ANALYSIS.

The tests that are used in examining a sample of water depend entirely on the objects for which the water is intended. Hence we may divide the tests into three different groups according to whether the water is to be used—

(*a*) For manufacturing or laundry purposes.

(*b*) For drinking purposes.

(*c*) For medicinal purposes, or for the extraction of certain valuable ingredients.

WATER FOR MANUFACTURING PURPOSES.

In a few industries certain special qualities are needed of the water supply, but, as a rule, waters under this head are valued according to their usefulness in steam boilers. When this is the case, it is necessary to examine for but few constituents. The chlorides are usually tested for to give a general idea of the amount of salt in the water, and then the water is carefully examined for the presence of the bicarbonates and sulphates of lime and magnesia.

These salts are objectionable because, on evaporation, they form crusts and deposits, which settle on the shell of the boiler, and not only diminish its evaporating power, but actually tend to shorten its life. The bicarbonates are less injurious than the sulphates, because, on heating, they change into carbonates, which precipitate as a fine mud, and do not at once adhere to the metal. But the sulphate of lime forms a hard, crystalline deposit which sticks closely to the shell and must be removed by hammer and chisel.

The bicarbonates, also, can be completely removed from water before entering the boiler, by precipitating them with lime water

or by heat, and then filtering or settling out the precipitates. To purify a water from the presence of sulphates, we can only add barium chloride, which removes the sulphuric acid, indeed, but leaves behind the lime and magnesia in the form of chlorides, which are often corrosive.

Water for Laundry Purposes.—The same lime and magnesia salts are objectionable when water is to be used for washing, for they combine with soap making sticky, insoluble compounds, which must first be formed and then be washed away before any cleansing will take place. This, of course, wastes both time and soap. A hard water, *i.e.*, water containing much lime and magnesia, can be softened by the addition of alkali, washing-soda or ammonia, not, however, without danger of injuring the clothes.

In this case, also, the sulphates are more troublesome than the bicarbonates, because they cannot be removed from the water by boiling, and hence make the water permanently hard.

Quantitative Determination of Hardness.—We can determine with some accuracy the comparative hardness of water by shaking a measured quantity of it with a standard solution of soap, and noticing how much soap is used up before a permanent lather is formed.

To make the standard solution, we usually prepare a strong solution of soap in hot dilute alcohol, and then reduce it to the proper strength, comparing it against a standard lime solution. The latter is made by dissolving one gramme of pure calcium carbonate in a little hydrochloric acid, evaporating off the excess of acid, and diluting to a litre with distilled water. For the strength described in the experiments, 10 c.c. of the lime solution should just balance 23.2 c.c. of the soap solution.

When making the test, the end of the reaction is distinctly marked by a change in sound and feeling as the mixture is shaken. The water no longer strikes sharp and clear against the glass, but both sounds and feels soft. The lime and magnesia soaps toward the end form a scum on the top, called the "false lather." The true lather, which should last for some minutes without breaking down, is soft and thick, and clings to the sides when the bottle is tilted.

To determine the permanent hardness, the water is again tested

after it has been thoroughly boiled. The difference between this and the total hardness obtained before, corresponds to the temporary hardness due to the bicarbonates.

TESTS ON DRINKING WATER.

We must now look for a different set of constituents, which shall cast some light upon the sanitary condition of a given water.

Biological Tests.

There are certain diseases—cholera, typhoid fever, dysentery, and the like—which are undoubtedly, in many instances, communicated by drinking water which has been contaminated by the excreta of previous patients. The injurious constituents of these waters are the specific germs of these diseases. Hence, the most satisfactory method of analysis, if it were practicable, would be to examine any suspected water for these dangerous microbes, and to let their presence or absence decide the question as to whether it should be used for drinking or not.

Unfortunately, with our present knowledge, this mode of analysis is hardly possible, even for one thoroughly skilled in such investigations. We have not yet isolated all the disease bacteria, nor can we always distinguish even those that we do know, and the identification of a few disease germs, especially in the presence of such a variety and such a number of harmless or even beneficial microbes as occur in water, is extremely difficult and, in many cases, practically impossible.

Counting the Microbes.—Efforts have been made, also, to determine the quality of a water by counting the germs present in it.

Methods.—This is at present done by the Koch gelatin plate-culture method, where a small amount of the water, $\frac{1}{4}$ or 1 c.c. as a rule, is intimately mixed with 5 or 10 c.c. of melted, sterilized, beef peptone gelatin, and the mixture is spread out and allowed to cool upon a cold sterilized glass plate. After standing from one to three or four days, the individual microbes develop into colonies, which are large enough to be seen and counted with the eye or with a low magnifying power. These colonies can then be isolated, and cultivated and studied by themselves.

Disadvantages.—This method of determining the number of the germs is not very troublesome, even when done carefully, but must unfortunately be considered far from accurate.

There is, to start with, some uncertainty as to whether the microbes have been so thoroughly mixed with the gelatin that each colony will be the outgrowth of an individual, and not of a group. Then, too, some of the germs grow rapidly, and cover over and obscure those of slower growth, some of which, indeed, can hardly develop in time to be counted.

Again, worse than this, it is impossible to tell how large a percentage of the microbes in water form colonies at all under these conditions. Some bacteria, as we know, need the presence of oxygen, and must be planted on the surface. Others, again, will not develop unless deep in the gelatin, away from the air. And, again, there is an indefinite number of microbes, some of which may be of great importance, which do not grow in gelatin at all, and hence are left out entirely. This fact has been brought into prominence during the last year or two, when the nitrifying ferments, which are universally present in natural water and which play such a very important part in its purification, have at last been isolated, and prove to be unable to develop in the ordinary solid culture media.

Significance.—But even if the number of germs could be accurately determined, it is doubtful if this knowledge would be of much importance in most cases. The quantity of bacteria present depends, often, far more upon the amount of filtration, of oxygen present, of exposure to air and to dust, and also, to an enormous extent, upon the comparative freshness of the sample, than upon actual contamination. And while it is agreed by all that the immense majority of microbes in water are not only harmless but actually beneficial, it is claimed by some authorities that the ordinary water bacteria are able to crowd out and destroy any stray disease germs that may find their way among them.

Without, however, going to extremes, it is safe to say that at present, with the methods now in vogue, the question of the purity or impurity of a water cannot be satisfactorily settled by biological tests alone.

Chemical Tests.

Although the disease germs themselves cannot be traced, still they are always accompanied by large quantities of excreta and other refuse organic matter—sewage, in short—two constituents of which, the nitrogenous organic matter and the chlorides, can be recognized even in minute quantities, and after considerable lapse of time. Hence, by carefully examining for these constituents, we are able to decide, not, indeed, whether a water is actually dangerous to health, but whether it is contaminated with material which may at some time or another contain disease germs.

Free Ammonia.—The organic matter in sewage, which is largely nitrogenous, is attacked almost immediately by the common putrefactive microbes, which oxidize the carbon and some of the hydrogen, and set free the nitrogen in the form of ammonia. Ammonia occurs in minute quantities in the atmosphere, and hence in rain and in all natural waters. But, excepting in mineral waters, where it is often quite abundant, it is rare to find perfectly pure waters containing much more than 0.0020 to 0.0025 grain to a gallon, or from 0.004 to 0.005 part per 100,000.

Nessler's Test.—To determine such minute quantities we make use of Nessler's solution, an alkaline solution of mercuric iodide, HgI_2, in potassic iodide. When a few drops of it are added to a dilute solution of ammonia or of an ammonium salt, it forms a deep red precipitate, which, even in very small amounts, imparts a yellow or brownish tinge to water. Thus:

$$2HgI_2 + NH_3 = \underset{\text{Mercur-Ammonium Iodide.}}{NHg_2I} + 3HI.$$

This precipitate, which may be considered as ammonium iodide with two mercury atoms in place of four atoms of hydrogen, is exceedingly heavy, one molecule of it weighing 541, as against 17 for one molecule of ammonia.

When using this test for quantitative work, the ammonia in a given amount of the water, 500 c.c. or so, is concentrated by distillation with a little alkali. Its quantity is determined by comparing the color that a volume of the distillate gives with a little Nessler's solution, with the color produced by the reagent

in the same volume of a very dilute standard ammonium chloride solution. This test, when used carefully, will determine accurately the presence of one part of ammonia in one hundred million parts of water.

Significance.—The amount of ammonia in a given water varies constantly, increasing with the amount of decomposing nitrogenous matter passing into it, and diminishing chiefly by being oxidized to nitrites and nitrates, and also, to some extent, by being directly absorbed by vegetation.

A water containing unusual amounts of it, especially if accompanied by albuminoid ammonia in any quantity, is undoubtedly open to suspicion of a contamination, the more dangerous because of recent origin.

Albuminoid Ammonia.—Besides the ammonia, there is always present, in fresh sewage or in recently contaminated waters, a certain amount of undecomposed nitrogenous organic matter. To get an idea of the quantity of this, we distil some of the water with the addition of an alkaline solution of potassic permanganate, and notice the difference between the amount of total ammonia thus evolved and of the free ammonia determined previously.

This difference we call albuminoid ammonia, and its quantity may be four or five times as much as the figures mentioned for free ammonia, without giving grounds for suspicion. When present in large quantities, however, it is a valuable indication of very recent contamination.

Nitrites.—After the nitrogenous matter has been sufficiently decomposed to liberate ammonia, another series of microbes, the so-called nitrifying ferments, begin to oxidize this ammonia, first into nitrites and finally into nitrates. Hence, the presence of the salts of nitrous acid in any quantity must be considered as indicating less recent contamination than ammonia, either free or albuminoid, and more recent than nitrates.

The test, however, is not as delicate as the Nessler's reaction; and, as the nitrites are probably a transition stage in the oxidation of nitrogenous matter, it is rather rare to get a good test for them in perfectly pure water. For this reason, probably, the significance of their presence has frequently been somewhat exaggerated.

The reaction is based upon the formation of a scarlet coloring matter, one of the "Azo" dye stuffs, by the action of nitrous acid upon two aromatic organic bodies. The test is interesting as being a counterpart of the well-known Ehrlich's reaction with urine of typhoid and other fever patients, where the nitrous and sulphanilic acids are mixed together and the compound corresponding to the naphthylamin salt is furnished by the urine.

Nitrates.—These substances which, as well as the nitrites, occur in small quantities in the air and in all natural waters, result from the final oxidation of the nitrogneous matter. Hence the nitrates rarely occur in large quantities in fresh sewage, but, in polluted waters, are still found after the free and albuminoid ammonia and the nitrites have all disappeared. They are themselves removed by vegetation.

It seems probable that, in this process of oxidation, not only are the foul smelling and tasting constituents of sewage all destroyed, but also the stray microbes, both harmless and dangerous, are crowded out and removed at the same time. Indeed, experience has shown, as in the case of the Thames above London, the Hudson below Troy, the Merrimack below Lowell, and many similar instances, that it is not unwholesome to drink water contaminated even with large quantities of sewage, provided sufficient time and opportunity be given it for self-purification. Still, the presence of nitrates in unusually large quantities, unless capable of special explanation, is always a suspicious circumstance, as showing contamination which, although at present probably harmless, may at some future time give rise to trouble.

Tests.—We can test for these substances in two ways, either by reducing them to ammonia and using Nessler's solution, or else by directly producing a colored compound.

(a) *Reduction Method.*—The best reducing agent is nascent hydrogen, which can be evolved in the water to be tested, either by dissolving aluminium in alkali, as in Lesson XIII., or else by the action of the water upon two different metals, such as zinc and copper. When these two, in close contact, are immersed in water, a true galvanic couple is formed, and the positive zinc is oxidized, and hydrogen is set free from the negative copper.

This hydrogen slowly converts the nitrates first into nitrites and finally into ammonia, according to the equations:

$$\underset{\text{Sodic Nitrate.}}{NaNO_3} + H_2 = \underset{\text{Sodic Nitrite.}}{NaNO_2} + H_2O.$$

$$NaNO_2 + 3H_2 = NH_3 + NaOH + H_2O.$$

This ammonia can be accurately determined by nesslerizing, and the quantity of nitrates calculated accordingly.

(b) *Phenol-Sulphonic-Acid Test.*—We can estimate the quantity of nitrates directly by noticing the depth of color which they produce in a solution of phenol in strong sulphuric acid. The reactions, which are similar to those in the fourth test in Lesson XIII., are as follows:

$$\underset{\text{Phenol.}}{C_6H_5OH} + H_2SO_4 = \underset{\text{Phenol-Sulphonic Acid.}}{C_6H_4.OH.SO_3H} + H_2O;$$

and then, in the presence of traces of nitric acid or nitrates,

$$C_6H_4.OH.SO_3H + HNO_3 = \underset{\text{Mono-Nitro-Phenol.}}{C_6H_4.OH.NO_2} + H_2SO_4.$$

The color is intensified by the addition of an excess of alkali.

Chlorides.—So far we have been discussing the nitrogenous derivatives of sewage, which must be considered as only temporary constituents of contaminated waters, diminishing and finally being removed, in process of time, by the action of natural causes. The albuminoid ammonia is oxidized to free ammonia, and this changes first to nitrites and then to nitrates, to be finally absorbed by plants.

The substances that we shall now test for do not tend to diminish after they have once entered a water, although the process of dilution may make their presence less apparent. Hence they may give evidences of contamination in a water after all the nitrogenous constituents have disappeared.

Derivation.—The chlorides, and especially the chloride of sodium, are universally present in natural waters, being derived both from the dried salt spray in the atmosphere and from the soil. The amount that is present in perfectly pure water varies enormously, depending largely upon the distance of the locality from the ocean, from saline springs, from beds of rock salt, and also, in general, upon the nature of the soil.

Besides this, common salt is always present in considerable quantities, not only in animal excreta of all sorts, but also in kitchen refuse, slops, and the like, which form such a large part of ordinary sewage.

Significance.—Accordingly, it is impossible, from the analysis of any one sample, to tell whether a largish proportion of chlorides is due to contamination or to perfectly natural causes. Nor can we lay down any general standard which shall be considered, in all cases, as the limit of the salt in unpolluted waters. But it is possible, with careful study of springs and water supplies that are known to be pure, to fix the average complement of chlorides in pure water for any particular locality. And any considerable excess, in adjacent water supplies, unless explained by special causes, can properly be deemed suspicious.

Determination. —It is easy to determine, with great accuracy, the quantity of salt present in water or in other neutral solutions, by using a standard solution of argentic nitrate, with a few drops of potassic chromate to act as an indicator. The red chromate of silver produced by the latter is decomposed into argentic chloride as long as any chlorides remain in solution. But directly the latter have been precipitated, the argentic chromate gives a red or orange color to the solution. Thus, as long as there are soluble chlorides present,

$$AgNO_3 + NaCl = AgCl + NaNO_3;$$

but when they have been all precipitated,

$$2AgNO_3 + K_2CrO_4 = Ag_2CrO_4 + 2KNO_3.$$

The standard solution, of the strength used and described in this lesson, contains 1 gramme of $AgNO_3$ in 401 c.c. of water.

Other Chemical Tests.—There are but few other tests worth mentioning in this connection.

In former years many efforts were made to determine the organic matter in water by noting its decolorizing power upon standard solutions of potassic permanganate. This reaction, however, of late years has been considered rather untrustworthy.

The presence of phosphates in any abundance is also a somewhat suspicious circumstance. These are always tested for with ammonic molybdate, using, generally, the residues from the evap-

oration of from 200 to 500 c.c. of the water. The presence of iron and of silica interferes with the reaction, and the latter, especially, ought to be removed before making the test, if any importance is to be attached to the result.

Again, in any important analysis we usually determine the amount of total solid matter, and, especially, of what is known as the organic and volatile matter. The former is obtained by evaporating a measured quantity of the water to dryness; while that part of the total residues which volatilizes at a dull red heat is set down under the latter name.

But, in general, from the tests already described, assisted, whenever it is possible, by careful examination of the source and surroundings, a chemist is usually able to determine with considerable accuracy whether a given sample of water is pure or is to be regarded with suspicion, if not actually condemned.

Analysis of Croton Water.—The following analysis of Croton water, made September 18th, 1891, and taken from the official report of the Health Department of New York City, is inserted here to show how a careful sanitary water analysis is reported.

Analysis of Croton Water.

September 18th, 1891.

A = parts per 100,000.
B = grains per United States gallon of 231 cubic inches.

	A.	B.
Appearance,	Slightly turbid.	
Color	Light yellow-brown.	
Odor at 38° C.,	Marshy.	
Chlorine in chlorides,	0.210 part.	0.122 grain.
Equivalent to sodium chloride,	0.346 "	0.203 "
Phosphates,	None.	
Nitrites,	Very faint trace.	
Nitrogen in nitrates and nitrites,	0.0408 part.	0.0237 grain.
Free ammonia,	0.0005 "	0.0003 "
Albuminoid ammonia,	0.0140 "	0.0082 "
Hardness, equivalent to carbonate of lime, Before boiling,	4.51 "	2.63 "
Hardness, equivalent to carbonate of lime, After boiling,	4.51 "	2.63 "
Organic and volatile matter (loss on ignition),	2.00 "	1.17 "
Mineral matter (non-volatile),	7.00 "	4.08 "
Total solids by evaporation at 110° C.,	9.005 "	5.25 "

This particular analysis was made at a time when, owing to an excessive drought and to other causes, the Croton water was more impure than it had been for a long time. This was shown by the presence of faint traces of nitrites, and, which was of more importance, by a decided increase in the amount of nitrates, as well as of the solid ingredients, both mineral, and organic and volatile. Thus, the official analysis of October 9th, 1891, shows that no nitrites could be recognized and that the nitrates had fallen from 0.0408 to only 0.0239 part in 100,000.

Still, judging even from this analysis, which certainly is distinctly less favorable than usual, the Croton water compares very favorably with the water supplies not only of foreign cities, but also of the other cities of this country.

MINERAL WATERS.

According to a well-known definition, a mineral water is a water containing either large quantities of the ordinary constituents or else some unusual constituent.

The ordinary constituents referred to are those that have already been discussed in the last five lessons; *i.e.*, the chlorides, sulphates, carbonates, nitrates, and phosphates of the metals iron, aluminium, calcium, magnesium, sodium, potassium, and ammonium. Small quantities of nitrites and of silica are also often present.

Of the unusual constituents, sulphuretted hydrogen, borax, the salts of lithium, and, in rare instances, free hydrochloric and sulphuric acids are the most interesting.

The tests for almost all these substances have already been discussed and need no further explanation here.

With regard to their relative importance when used for medicinal purposes, but little can be definitely said. Indeed, the therapeutic effect of a mineral water seems to depend as much upon the influence it has upon a patient's imagination and upon the changes in diet, exercise, occupation, and in fact in general hygiene, that it forces on him while "taking the cure," as upon any particular constituent.

Still, it may be well to remind the student that the sulphates of soda and of magnesia, and the bicarbonate of magnesia have

decided laxative properties; that the bicarbonate of soda is of value as a mild alkali; and that the carbon dioxide acts as a mild and useful stimulant to both stomach and intestines. The sodium chloride is chiefly valuable for flavoring the water, while it is very doubtful whether the iron and even the sulphuretted hydrogen have any special tonic effects, when taken in this form.

The bromide and iodide of sodium, and the different salts, principally the bicarbonate, of lithia, for which such remarkable properties are claimed, occur in mineral waters in such minute quantities, or mixed with such large amounts of other salts, that they can be of no special service. Indeed, the natural lithia waters, which are at present so largely advertised, rarely contain more than infinitesimal quantities of the salt, and in many cases contain none at all.

LABORATORY EXPERIMENTS.

WATER ANALYSIS.

I. Water for Manufacturing and Laundry Purposes.

Qualitative Tests.—Half fill three test-tubes with well-water, and test them as follows:

1st. *For Chlorides.*—Add one or two drops of HNO_3 dil. and one or two drops of argentic nitrate, $AgNO_3$ = ppt. of AgCl.

2d. *For Sulphates.*—Add one or two drops of HCl dil. and one or two drops of baric chloride, $BaCl_2$ = ppt. of $BaSO_4$.

3d. *For Lime.*—Add one or two drops of NH_4OH and NH_4Cl; then add a little ammonic oxalate, $(NH_4)_2C_2O_4$ = ppt. of CaC_2O_4.

Repeat these tests with Croton water, and notice that in this the ppts. are almost imperceptible, although quite distinct in the well-water.

Quantitative Determination of Hardness.—Place in a stoppered bottle 100 c.c. of Croton water and add, from a burette, some "standard soap solution," shaking well after each addition. Stop when a permanent lather is formed, and when, on shaking, it sounds and feels *soft*.

Each c.c. of soap solution used is equal to a quarter of a grain of $CaCO_3$ in one gallon of the water.

Repeat this test with 50 c.c. of the well water. Notice the formation of a "false lather" of lime or magnesium soap before the true *soft* lather. With this quantity of water each c.c. of soap solution corresponds to half a grain of $CaCO_3$ per gallon.

II. Drinking-water.

1st. *Free Ammonia.*—Nearly fill a large test-tube with well-water and add a few drops of Nessler's solution. A yellow or brownish color indicates free ammonia.

Repeat this test with Croton water; the color will not turn at all.

To some Croton water in a test-tube add one drop of a very much diluted NH_4OH solution; then add a little Nessler's solution, and notice the decided change of color.

2d. *Nitrites.*—Fill two large test-tubes respectively with C o-ton and with well water. To each add a few drops of the saturated solutions of sulphanilic acid, and of naphthylamin hydrochlorate. A pink or red coloration appearing in a few minutes shows the presence of nitrites.

Notice that the Croton water does not turn pink until it has stood for some time, and has absorbed some nitrites from the air.

3d. *Nitrates.*—(*a*) *Reduction Method.*—Clean the zinc in the wide-mouthed bottle by adding some water and a little HCl dil., letting it effervesce for a minute or two, and then rinsing it out thoroughly. After this, cover it with water, add three or four drops of $CuSO_4$, and let it stand for a few minutes until the zinc is fairly covered with a black deposit of metallic copper. Then rinse it out well, and fill the bottle with Croton water.

Test the reducing action of this "zinc-copper couple" on nitrates by adding two or three crystals of $NaNO_3$ to the water in the bottle, shaking it until they dissolve, and then letting it stand quietly until the end of the hour. Notice the slow but continuous evolution of hydrogen, and, before leaving, test the solution for *nitrites* and for *free ammonia*, as described above.

(*b*) *Phenol-Sulphonic-Acid Test.*—Add one or two drops of Na_2CO_3 to 50 c.c. of the well-water, and evaporate the mixture to dryness in an evaporating-dish. This can be done at first over the flame, but must be finished over the water-bath. Cover

the residue with a solution of phenol-sulphonic acid, made by dissolving, carefully, in a test-tube, a few drops of phenol with twenty times its bulk of common sulphuric acid. Then add about 10 c.c. of Croton water and an excess of KOH.

If nitrates are present in the well water, the mixture will have a yellow or even an orange color.

4th. *Chlorides.*— (*Quantitative Determination.*)— Measure 50 c.c. of Croton water into a beaker, and add one drop of potassic chromate, K_2CrO_4. Now run in very carefully, from a burette, the "standard $AgNO_3$ solution," stirring constantly with a rod. Notice how the red ppt. of Ag_2CrO_4, which is formed by each drop of the $AgNO_3$ solution, dissolves when it is mixed in with the yellow liquid, and is converted into a white or yellowish cloud of AgCl.

When the red ppt. dissolves slowly and with difficulty, add the $AgNO_3$ solution only a drop at a time, stirring well after each addition, until the color of the mixture just changes from yellow to orange or orange red. Then stop, read the burette, and the number of c.c. of standard solution used (for this quantity, 50 c.c., of water) will equal the number of grains of NaCl in one gallon of the water. Repeat this test with the well-water.

III. Mineral Water.

Test the sample of mineral water as follows:

1st. *Bicarbonates of Na, Ca, and Mg.*—(*a*) Notice that the water is alkaline to test papers, after it has been boiled for a minute or two to expel the CO_2 gas.

(*b*) Add some acid to the water. Notice the effervescence of CO_2 gas.

(*c*) Boil some of the water in a beaker for 5 or 10 minutes. Notice the white deposit of $CaCO_3$ and $MgCO_3$, which dissolves with effervescence in acids.

2d. *Chlorides, principally Sodium Chloride.*—Determine the amount of NaCl in the water, as described above, using, however, only 5 c.c. of water for the test, diluting it with a little Croton * water. In this case the number of grains of salt per gallon is found by multiplying by 10 the number of c.c. of solution used.

* *N.B.*—Distilled water would give more accurate results.

3d. *Sulphates.*—Test for these with HCl and $BaCl_2$. In most of the common mineral waters this test will be very faint.

4th. *Iron.*—In siphon waters and in most of the table mineral waters this element will not be present. When testing Saratoga waters, however, or any others where even small traces of iron are present, it is generally possible to notice the brownish flakes of $Fe_2(OH)_6$ floating in the water.

To prove its presence, add enough HNO_3 to make the water slightly acid, warm for a minute or two, and test for Fe(ic) with NH_4CNS.

5th. *Calcium and Magnesium.*—Add NH_4OH, NH_4Cl, and $(NH_4)_2C_2O_4$ to some of the water = ppt. of CaC_2O_4. Warm, filter carefully, and test the filtrate for Mg with Na_2HPO_4.

6th. *Lithium.*—Acidify the water with HCl, and test for Li by the flame test, as in the last lesson.

In practice it is usually necessary to concentrate the water by evaporation before making this test, and also to confirm the results by the spectroscope.



PART VI.

ANIMAL TISSUES AND SECRETIONS.

LESSON XVII.

BONE

Composition.—The chemical composition of bone varies considerably, according to the age and health of the individual and of the bone itself. In general, we may say that fresh human bone from healthy, well-grown individuals contains on an average about 50% of water, 16% of fat, 12% of ossein, and 22% or so of mineral matter. The fat is almost all contained in the yellow marrow, which is found in the medullary cavity. Small quantities of it occur also in the red marrow, which is chiefly found at the ends of the long bones, as well as in short bones and in some of the bones of the skull. Besides fat, the marrow, both yellow and red, contains cholesterin, salts, and nitrogenous matter consisting of albuminoids, proteids, and small amounts of the so-called "extractives," while in the red marrow, which is supposed to be a source of the red blood-corpuscles, we find a little hæmoglobin.

If the bones are first carefully dried and then extracted with ether to remove the fat and marrow, we have left an intimate mixture of mineral matter with a peculiar, tough, albuminoid substance described by various authors under the name of ossein or collagen. This organic material seems to give elasticity to the bone, while the mineral constituents furnish strength and hardness.

The comparative amounts of these two vary considerably according to age, the mineral matter increasing in quantity as the individual develops. Hence, in young children the bones are more elastic and yield more or less to pressure, while in old age the percentage of mineral matter rises so high that the bones are apt to become brittle. In certain diseases, also, such as osteomalacia for example, the proportion of mineral matter diminishes considerably, so that the bones may soften or become pliable. On the other hand, small doses of phosphorus and also of arsenic

tend to make the bone more compact and to increase the inorganic constituents. In general we can say that of dry, clean bone about one-third is organic and two-thirds inorganic. The exact figures, taking the average of several analyses of Zalesky,* are, for human bones 34.56 and 65.44%, and for beef bones 32.02 and 67.98% respectively.

Ossein (Collagen).—This substance, which is very possibly a mixture of several albuminoids, and about which, as yet, very little is known, can be readily separated from the earthy matter by macerating dry, clean bones for some hours in dilute hydrochloric acid. It is an evidence of how intimately the two are associated that a bone treated in this way still retains its original shape, although all the mineral constituents have been dissolved out. The residue is, when wet, a soft, translucent, flexible, nitrogenous body. It is insoluble in cold water, dilute acids, and alkalies, but when boiled in water it slowly dissolves, and is converted into the substance known as gelatin or glue.

Gelatin.—This material, which is similar to and, possibly, identical with that obtained by boiling connective tissue, tendons, hoofs, epidermis, fish scales, and similar substances, dissolves with ease in warm water and swells more or less in cold water. Its solutions, if not too dilute, have the curious property of forming a jelly on cooling, a property, however, which is lost after continued boiling.

When very carefully prepared, it is colorless or faintly yellow, transparent, and tasteless, and is largely used in food preparations. Its value for food has been much discussed, and at one time it was generally believed that the nutritive power of gelatin was extremely slight. There is no doubt, however, that it can be digested and will replace other food stuffs, although it cannot entirely take the place of proteid matter. An impure form of gelatin, called glue, is largely used for adhesive purposes. A mixture of gelatin with bichromate of potash is also employed in some of the modern photo-mechanical processes, being rendered totally indifferent to either hot or cold water by exposure to light.

It will be noticed, in the tests for ossein and gelatin, that, while they respond to some of the general proteid reactions, they are

* Hoppe-Seyler's "Medic. Chem. Untersuchungen," p. 38.

not affected by them all. Tannin or tannic acid has the property of forming with these, and with other albuminoids, an insoluble compound which is decomposed with much difficulty, and the presence of which constitutes the difference between raw hide and leather.

The chemical composition of both ossein and gelatin is about the same as that of other albuminoids, namely, according to Frémy, * C 50, H 6.5, N 17.5, O and S 26%. When bone, or any other nitrogenous animal product, is subjected to dry distillation, these different elements are driven off, combined with each other in varying proportions. Water is the first constituent to make its appearance, being volatilized at about 100° C. Then the nitrogen begins to come over, combined with hydrogen in the form of ammonia, while the oxygen combines with carbon and also with some hydrogen to form CO_2, CO, and H_2O. At the same time hydrogen and carbon distil over together, at first in the form of light volatile hydrocarbon gases, CH_4, C_2H_6, and others, then as heavier members of the fatty and also of the aromatic compounds, and finally as dark, thick, tarry bodies, principally aromatic. The carbon that still remains is left behind as coke or charcoal.

Bone Black or Bone Charcoal.—The residue from the dry distillation of bone differs from ordinary animal or vegetable charcoal in many important respects. In the first place it contains very little carbon, only from 6 to 12%, the rest being composed of mineral matter. And in the next place, owing, it is supposed, to its great porosity and to the immense surface it thereby offers, it has a wonderful power, when used as a filtering medium, of extracting from liquids substances not only in suspension, but even in solution. These latter may be inorganic, like the salts of a great variety of different metals, or organic, such as carbohydrates, alkaloids, and especially various coloring matters. It has been claimed that this power may be due to the action of the gases, oxygen and carbon dioxide, condensed and stored up in the pores of the charcoal. This, however, seems hardly a satisfactory explanation.

The absorbing and filtering capacity of bone black does not

* Jahresber. u. d. Fortsch. d. Chemie, 1854, p. 701.

last indefinitely, but, curiously enough, after this power has been lost for one class of compounds, it still holds good for others. For instance, bone black which has exhausted its power for removing salts can still be used for decolorizing, and *vice versa*. Bone black which has lost its special filtering power can be restored to almost its original condition by thoroughly washing it, dissolving out the absorbed lime with very dilute acids, removing the retained organic matter by alkalies or by fermentation, rewashing in boiling water, carefully drying, and heating to redness. There is always a certain waste, however, and the pores become gradually filled with lime, or with coke from the organic matter, till finally, after fifteen or twenty treatments, the bone black becomes useless.

Large quantities of bone black are used in the refining of sugar, the manufacture of glucose, and in many other important industries. In the laboratory it is frequently used to decolorize solutions, as, for instance, in preparing a sample of urine for the polariscope.

Bone Ashes.—The mineral matter of bone is best obtained by burning off the organic material. Its composition varies more or less, but, according to Zalesky's * analyses, the ash of normal human bone contains, on an average, calcium phosphate 83.89%, magnesium phosphate 1.04%, calcium carbonate 13.38%, with small quantities (0.18% and 0.23% respectively) of chlorine and fluorine. According to some experimenters it is possible to replace a portion of the calcium in bone by magnesium, aluminium, or even strontium, by dieting animals on food rich in salts of those metals.

When making qualitative tests for lime and magnesia in bone ashes, we are embarrassed by the presence of phosphoric acid. The so-called earthy phosphates are insoluble in alkaline solutions. Hence, if we should add enough ammonic hydrate to change the reaction, as in all our previous separations of the two metals, both the calcic and magnesic phosphates would be precipitated together without waiting for the addition of ammonic oxalate or sodic phosphate. To get rid of the phosphoric acid without precipitating the Ca and Mg at the same time, we add a considerable quantity of ferric chloride, more than enough to sat-

* Loc. cit.

isfy all the phosphoric acid present, and then neutralize the solution with baric carbonate. The iron at once precipitates as phosphate, carrying down all the phosphoric acid, and then, after getting rid of the dissolved barium with a little ammonic sulphate, we can proceed to separate the Ca and Mg as in previous lessons.

When examining the ashes of other animal tissues than bone, we can recognize the presence of iron, derived from the hæmoglobin of the blood, and also of somewhat greater amounts of chlorides. In other respects the tests will be the same.

LABORATORY EXPERIMENTS.

BONE.

I. **Dry Distillation.**—Fill a test-tube nearly full with pieces of dry bone. Fit to it the delivery-tube, heat it gently over a burner, and notice the gradual decomposition of the bone. At first water distils over, with some fumes. Test these for NH_3, with wet test-paper, and by holding near them a rod dipped in HCl conc. (*v.* Lesson XV.). Continue heating, and a combustible gas will come over. Ignite this at the end of the tube, and notice that it burns with a bright yellow flame, like coal gas. After this there will distil over a heavy, dark-colored, strongly smelling liquid, like coal-tar, and finally a residue will be left in the test-tube, consisting of bone charcoal or bone black.

II. **Bone Black.**—Fill the funnel-tube nearly full with granular bone black, rinse it once with water, and then filter through it again and again, a little very dilute solution of indigo.

Keep some of this same indigo solution for comparison, and notice how the bone black gradually decolorizes it.

III. **Bone Ashes.**—Heat several small pieces of bone, one after the other, on charcoal, with the blow-pipe. Notice that at first they turn black, and give off fumes, but that after longer ignition they turn white again. Now drop them into a little HNO_3 dil. in a small beaker; warm, and test the solution as follows:

1st. *Carbonates.*—Notice the effervescence of CO_2 from each piece when it enters the acid.

2d. *Phosphates.*—Fill a test-tube two-thirds full of $(NH_4)_2MoO_4$, and add a few drops of the solution = yellow crystalline ppt.

3d. *Chlorides.*—Test for these by adding a drop of $AgNO_3$ to a good deal of the solution in a test-tube. Notice that, if present at all, they are present in very minute quantities.

4th. *Calcium and Magnesium Phosphates.*—Put some of the solution in a test-tube and add NH_4OH = white ppt. of the so-called "earthy phosphates."

5th. *Separation of Ca and Mg in the Presence of Phosphates.*—To all the remaining solution, in the small beaker, add slowly some Fe_2Cl_6. After every few drops test the mixture by adding a drop of it to some NH_4OH in a test-tube. When the ppt. thus formed is *yellow*, and not *white*, stop adding Fe_2Cl_6, and add Na_2CO_3 until the ppt., formed as the latter enters the mixture, is only just redissolved when stirred up with the rest of the liquid, *i.e.*, until the mixture is nearly neutral. Then add half an inch of $BaCO_3$, warm, and filter off the Ba and Fe(ic) phosphates. To the filtrate add a quarter of an inch of $(NH_4)_2SO_4$, boil, and filter off the $BaSO_4$ through a double filter. To this filtrate add a little NH_4OH and then $(NH_4)_2C_2O_4$ = ppt. of CaC_2O_4. Filter, and test for *Mg* in the filtrate with Na_2HPO_4.

Repeat these tests with ashes from the cremating-furnace, dissolving them in a small beaker with hot HNO_3 dil., filtering, and testing the filtrate as above. Notice that these ashes contain more chlorides than the bone ashes, and also give a reaction for iron with NH_4CNS.

IV. **Ossein (Collagen).**—While making these last tests boil the piece of "macerated" bone, for half an hour or more, with a little water in the saucepan. Notice that the bone disintegrates and even dissolves. Test the solution as follows:

1st. By the xantho-proteic, biuret, and Millon's reactions.

2d. Notice that it forms ppts. with (*a*) picric acid, (*b*) $HgCl_2$, (*c*) a solution of tannic acid.

V. **Gelatin.**—Place the glue in a little warm water in a small evaporating-dish. Warm very gently over a water bath and notice that the glue gradually dissolves. Put some of this solution in a test-tube and let it cool. Notice that, if sufficiently concentrated, it will gelatinize. Put some of the same solution

in another test-tube and boil it hard for a few minutes. Notice that, even if concentrated, it will no longer gelatinize.

Make the same tests on this solution as on the solution of collagen, above; also—

1st. Add some to a a little alcohol in a test-tube = ppt.

2d. Notice that it is not ppted by either alkalies or mineral acids.

3d. Notice that it is not ppted by acetic acid and potassic ferrocyanide.

LESSON XVIII.

MILK.

This may be described as a secretion from the breasts of female mammalia which begins a few days after parturition, and is intended to serve as sole nourishment for the infant offspring. The secretion, which begins at the end of pregnancy and continues until it is replaced by milk, is known as colostrum. It differs very materially from milk in that it contains from 25 to 30% of



FIG. 5.—COLOSTRUM, FROM A HEALTHY LYING-IN WOMAN, TWELVE HOURS AFTER DELIVERY (FUNKE).

solid matter, mostly belonging to the proteid group, and is full of little cells, known as colostrum corpuscles, which more or less resemble in appearance the white blood cells.

Properties.—Milk is a white or light yellow, opaque liquid, with a specific gravity varying, under different conditions, from 1.018 to 1.045. It has a reaction not far from neutral, and indeed is usually amphoteric, i.e., alters the color of both red and blue test paper. It is claimed that this is due to the simultaneous presence of both alkaline and acid sodium phosphates.

Composition.—Milk may be considered as a perfect food. In

other words, it contains representatives of all the classes of proximate principles already discussed, in the proportions best suited to support life. It is an aqueous solution in which are suspended fat globules, casein, and, for some little time after birth, a few colostrum corpuscles. We find dissolved in it milk-sugar, three if not four different proteids, various mineral salts, traces of lactic and acetic acids, of alcohol, lecithin, cholesterin, urea, and creatin and also the gases oxygen, carbon dioxide, and nitrogen.



FIG. 6.—HUMAN MILK GLOBULES, FROM A HEALTHY LYING-IN WOMAN, EIGHT DAYS AFTER DELIVERY (FUNKE).

Its composition varies very considerably, not only with the species of animal, but with the breed and also with the individual. Besides this, from the same individual the milk varies in quality according to the state of health, mental as well as physical, the food, exercise, time of day, length of time since previous milking and since parturition, and many other conditions.

Cow's Milk.—This milk is so universally used that its composition is a matter of considerable interest. The following figures, given by König,* represent analyses made upon several hundred different samples.

	Water.	Casein.	Albumin.	Fat.	Sugar.	Salts.
Minimum,	80.32	1.79	0.25	1.67	2.11	0.35
Maximum,	90.69	4.23	1.44	6.47	6.03	1.21
Average,	87.17	3.02	0.53	3.69	4.88	0.71

Human Milk.—Breast milk differs from cow's milk in two or

* J. König, "Zusammensetzung der Menschl. Nahr. u. Genussmittel," Berlin, 1889, p. 295.

three particulars. It contains considerably more lactose, and less casein. The casein, too, is different from that of cow's milk; it coagulates less readily and in lighter flocks, and is decidedly more digestible. It is probably on account of this constituent that, in spite of all precautions, it is sometimes almost impossible to make a delicate nursling thrive upon cow's milk.

The amount of fat is about the same. Hence it is advisable, when cow's milk must be given to infants, to dilute it with water, so as to reduce the amount of casein, and then to add both sugar (milk-sugar if possible) and a little cream. The following figures are taken from a series of analyses made with great care and according to modern methods, by Professor Leeds,* upon eighty samples of milk from normal women, whose histories were taken at the same time:

	Water.	Proteids.	Fat.	Sugar.	Ash.	Specific Gravity.
Minimum,	83.21	0.85	2.11	5.40	0.13	1.026
Maximum,	90.08	4.86	6.89	7.92	0.37	1.035
Average,	86.732	1.995	4.131	6.936	0.201	1.0313

Accordingly, in round numbers, we may say that normal breast milk has an average specific gravity of 1.031, and contains about 2% of casein and other proteids, 4% of fat, 7% of sugar, and 0.2% of ash.

Another difference between cow's and breast milk, which is especially important with respect to the feeding of young children, depends upon the fact that the breast milk is fresh and sterile when taken, while the cow's milk always contains micro-organisms, and is frequently used when in a state of incipient decomposition. Milk is an excellent culture medium for almost all kinds of microbes, which increase in it with great rapidity unless the temperature is kept very low. Two Austrian authorities, Escherich and Cnopf, have stated that they have found, by the already described plate-culture method, over a million bacteria and other germs to the cubic centimetre of milk as it is ordinarily handled in summer. Other observers report the finding of five or six hundred times as many microbes in milk that has stood at the ordinary temperature of a room for twenty-four

* Jour. Amer. Chem. Soc., vol. vi., pp. 253–280.

hours. These germs belong almost invariably to the ordinary putrefactive, and hence harmless, varieties. But still, when present in such enormous quantities, they, or at any rate their products, must have some deleterious action upon the digestive systems of delicate children. For this reason, wherever milk cannot be obtained perfectly fresh, it is now considered a necessary precaution to thoroughly sterilize it before feeding it to infants.

Proteids in Milk.—Of the different albuminous substances the casein, which has already been mentioned, is by far the most important. Besides this, there is a little albumin, either the same as or very similar to serum albumin, and also minute quantities of a globulin much like the paraglobulin of the blood. It is also claimed that still another proteid, quite similar to the albumoses and called lacto-protein, is present in small quantities. This, however, may be a decomposition product derived from the others.

Casein.—The casein is not dissolved in the milk, but is held suspended in it in a thin, loose, swollen condition. It can, in fact, be filtered out by straining milk through clay filters. The casein of cow's milk can be prepared pure, by precipitation from diluted milk with very weak acetic acid, and then by frequent solution and reprecipitation with dilute alkali and acid.

When pure, it is a fine white powder, leaving practically no ash on burning. It is insoluble in water, although it reddens moist blue litmus paper. It dissolves readily in alkalies, and the solutions do not coagulate on boiling, but form a scum on the surface. The scum, however, noticed in boiled milk may perhaps be due to the coagulation of some of the albumin. Casein can be precipitated from its solution by an excess of salt or by dilute acids, but it dissolves quite readily in stronger acids, especially in hydrochloric.

Casein is readily coagulated by acids and by the peculiar unorganized ferment known as rennet, or, from the German, "lab." In every case the coagulation seems to depend upon the presence of phosphate of lime, although exactly how is not known. Rennet is present in the stomachs of all infant mammalia, and is extracted on a large scale for use in the manufacture of cheese. One part of moderately pure ferment is able to coagulate several

hundred thousand parts of casein. When casein is coagulated by the ferment, it seems to produce, at the same time, small quantities of a soluble proteid which remains in the whey and i known as whey albumin.

Milk-Sugar.—This carbohydrate has already been discussed under Lesson IV. Its principal point of difference from the other sugars and from the glucoses is the fact of its fermenting to lactic acid. This occurs spontaneously when unsterilized milk is allowed to stand at an ordinary temperature. The acid thus produced is strong enough to cause coagulation of the casein. The reaction is supposed to be one of hydration, as follows:

$$\underset{\text{Lactose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Lactic Acid.}}{4H.C_3H_5O_3}$$

It is always accompanied, however, in the case of milk, by a slight evolution of CO_2, and by the production of small quantities of other compounds, probably owing to the presence of other ferments.

The true lactic fermentation is due to different varieties of bacteria, some of which, as, for instance, Bacillus acidi lactis, have been carefully studied. It takes place most rapidly at a temperature of 30 to 40° C. and entirely ceases when the milk is cooled to 2 or 3° C. It is of course hindered by the various antiseptics.

Milk is such an excellent culture medium, however, for microbes of all sorts that it not infrequently undergoes other fermentations as, for instance, turning blue under the action of Bacillus cyanogenus, turning slimy, or cheesy, or bitter, or, more important than any of these, developing the poisonous ptomaine tyrotoxicon. This latter, the active agent in the poisonous milk, cheese, and ice cream, of which so many cases are constantly occurring, is undoubtedly caused by the growth of some microbe not yet identified. Its symptoms resemble very closely those produced by poisonous mushrooms. They are constantly being mistaken for the symptoms of an acute metallic poison, such as copper or arsenic, but are much less permanent in their effects.

Inorganic Constituents.—The mineral salts of milk are but small in quantity. The most important of them is the phosphate of calcium. Besides this we find present the phosphates and

chlorides of potassium and sodium, with small quantities of sulphates, and also of magnesium salts. There is the merest trace of iron, probably contained in the yellow coloring matter of the fat.

Butter Fat.—This has already been described under Lesson VII. It occurs in milk in the form of small globules, varying in size from 0.0015 to 0.01 mm. Besides the fat, these globules contain small quantities of cholesterin and lecithin, and also a little yellow coloring matter which seems very similar to the lutein from the yolk of an egg.

It has often been suggested that each globule is enclosed in a little bag of proteid mattter which must be burst in the process of churning, before the butter can collect in lumps. One point in favor of this view is the fact that it is extremely difficult to extract the fat from milk by shaking it with ether, without the previous addition of alkali or acetic acid; and also that, if it is extracted without such addition, the liquid is not perfectly clear, but still looks turbid and almost opaque.

It is at present generally believed that the butter is simply emulsified. This emulsion is a particularly perfect one, however, owing to the supended casein, which, in the absence of the fat, would itself makes the milk more or less opaque. According to this theory, the action of the alkali or acid is not to dissolve up the bags from the fat globules, but simply to thin down the casein and alter its emulsifying power.

Adulterations of Milk.—Of the various substances that have been mentioned as actual or possible adulterants of milk, but few are met with in practice.

Sometimes we find samples of milk to which some chemicals have been added as preservatives. Salicylic acid is occasionally used for this purpose, but usually either carbonate of soda or borax is employed, the latter not only acting as an alkali to neutralize the lactic acid, but having some slight antiseptic properties as well. These latter substances are readily detected, on analysis, by igniting a measured sample of milk and noticing the weight of the ash.

The two common ways, however, of falsifying milk are, first, by skimming, and second, by the addition of water.

The sale of skimmed milk instead of whole milk is particularly

objectionable. It not only deprives milk of a valuable constituent, but, which is of more importance, it usually delays the delivery of the milk at least twelve hours and frequently more, thereby greatly increasing the danger of decomposition.

The addition of water, which is exceedingly common excepting where close supervision is kept by the proper authorities, is a fraud indeed, but is less dangerous to health. In some few cases, however, the use of contaminated water for adulteration and even, innocently, for washing the cans, has caused outbreaks of typhoid fever and other diseases.

THE TESTING OF MILK.

The method to be employed will depend on the object for which the tests are made, whether for the municipal regulation of the milk traffic or for the determination of the comparative quality of a sample of milk, as, for instance, in the clinical examination of the milk of mothers and nurses.

The Municipal Regulation of the Milk Traffic.—In this case the inspector must obtain results that will enable him to appear as a witness against the dealer, and establish the fact that the milk is not the unadulterated product of the cow. This conclusion is best reached by examining the sample with a specially constructed specific-gravity instrument, or hydrometer, known as the lactometer.

The Lactometer.—The two fixed points on the scale of this instrument are the 0° mark, which is at 1.000, the specific gravity of pure water, and the 100° mark, which is set at 1.029, the *minimum* gravity of milk. The graduations are continued to 120° and 130°. The point 1.029, which was long ago fixed in Europe as the limit of the density of genuine healthy milk, was redetermined, in 1875–76, by the health authorities of New York and New Jersey, from actual experiments at the dairies. Out of 1,600 cows whose milk was examined, only six, two of whom were sick at the time, were found to give milk below that figure. Of late years, owing to the introduction of cattle which, by special feeding, can be made to give large quantities of very poor milk, the number of specimens of milk below this standard has increased. But such milk is also below any standard that has yet been introduced, and

is still so rare that it is never found in cans containing the mixed milk of several cows. Hence it is still the truth that a sample of milk taken in the city, which stands at less than 100° on the lactometer, cannot be genuine cow's milk.

But besides the specific gravity, the examiner is expected to notice both the color and the consistence of the liquid as it drops from the lactometer on taking it from the milk. The bulb of the instrument is filled with shot, which furnish a black background for the thin film of milk; and, with a little practice, even an ill-educated person can use the instrument correctly.



FIG. 7.—THE LACTOMETER.

Genuine milk stands above 100°, generally from 105° to 120°, and is opaque and of good consistence.

Skimmed milk is heavier than whole milk, and hence will stand even higher on the lactometer. It is distinguished, however, by being thin and watery.

Watered milk will have a light specific gravity, and, if it is watered enough, will stand below 100° on the scale, and be at the same time thin and watery.

Cream will also stand low on the lactometer, but will at once be recognized by its color and consistence.

Moderate skimming or watering, of milk originally of good quality, cannot be detected by this or any other known method; but when the watering has been so great as to lower the specific gravity even 1° below the 100° mark, the milk can be condemned with confidence, upon the evidence of this instrument alone. The great advantage of this test lies in the fact that not only can the quality of the milk be accurately determined, but also that the instrument is cheap, and the test can be easily and rapidly made by the farmer, the dealer, the inspector, or the consumer.

Owing to the fact that cream is lighter than milk, it has been suggested that milk very rich in cream would therefore be light, and be liable to be condemned by the lactometer.

This objection might have some basis if the cream were added to ordinary milk. But apart from the fact that in New York, at least, it is not customary to adulterate milk with cream, the lactometer does not only show the weight but also the color and consistence of the milk, and one glance at the bulb, after the inspector had withdrawn his instrument, would at once prevent such a mistake.

Natural rich cow's milk, however, like that from the Jersey cattle, for instance, is, as a rule, not light but heavy. For it is rich not from an excess of fat only, but from a diminution in the amount of water; and the increased quantities of casein and sugar more than counterbalance the loss of weight due to the cream.

Quantitative Analysis.—This method is often resorted to in order to determine adulteration, but it at once introduces a new set of difficulties. The composition of genuine milk is just as variable as its specific gravity, and it is even more difficult to establish a natural minimum for each of the different constituents. In fact, it is found necessary, where analysis is depended upon, to fix the minima by law. Many States have fixed the minima at 12% for the solids left on evaporation, 3% of which must be fat. Other States have put the total solids at 11.5, 12.5, or even 13%, the fat at 2.5, 3.5, 3.66, or 4%, and the ash at 0.60 or 0.70%.

There is also considerable difference of opinion as to the most reliable methods of analysis, and the results obtained by various analysts, working with different methods, rarely coincide.

A very serious objection to analysis is the fact that the test cannot be made on the spot, but must be executed in the laboratory, by a skilled chemist, using expensive apparatus and with the expenditure of a good deal of time. This raises the cost of the test above the value of the milk, so that neither producer nor dealer can afford to have it made, while the delay in obtaining the results robs the inspection of much of its force. If a particular sample should have a composition below the legal limits, the milk has still been distributed among the consumers; while

if the milk is shown to be watered by the lactometer, it can and should be poured into the gutter at once. In the case of skimmed milk, however, where the liquid, although standing well above 100° on the lactometer, looks suspiciously thin and blue, it is often advisable for the inspector, before condemning the sample, to have his suspicions confirmed by analysis or, although less accurately, by the cream test.

The Percentage of Cream.—It has often been suggested that the quality of a sample of milk could be determined by noting the amount of cream that rises in a given time. This can be easily done by letting the milk stand over night, or for twenty-four hours if necessary, in a graduated cylinder.

This method unfortunately, although often useful, especially when made in connection with the specific-gravity determination, is too unreliable for municipal inspection. The cream from different kinds of cow's milk differs very much in consistence and in composition, and a milk containing but little fat may actually furnish more cream than milk containing twice that amount.

This will be seen at once from the following examples, given by H. Schroeder:

Samples No.	1.	2.	3.	4.	5.	6.
Fat,	3.54%	4.87	4.09	5.38	3.13	4.09
Cream,	21. %	16.	10.	10.	12.	13.

Other Tests.—Various tests have also been suggested which depend solely upon the color or the opacity of the milk to be examined. It is, of course, a fact that the appearance of the milk varies with the amount and quality of the cream, and, to a slight extent, with the quantity of casein contained in it. But while it is possible to distinguish in this way a sample of watered milk from cream, or of skimmed milk from good whole milk of the same specific gravity, the difference between individual samples of whole milk and watered milk is so slight that no sharp dividing line can be made between them.

Clinical Tests for Breast Milk.—This very interesting subject has, until very recently, received almost no attention in this country, although it is of great importance in connection with the digestive disorders of nurslings.

It is well known that no artificial food, however carefully pre-

pared, can quite take the place, for young infants, of the mother's milk. Yet in case after case it happens that the milk, for some reason or another, does not agree with the child, and hence that a new nurse must be obtained, or else that the child must be weaned and brought up by hand, often with considerable risk.

Careful investigation has shown that when the milk disagrees with a child, there is almost always some decided variation in the composition. The percentage of sugar usually remains about the same, but the fat and the casein vary considerably in the milk from the same woman, with the condition of the nervous system, and also with changes of diet, exercise, or of general hygiene. When these variations are recognized, it is often possible, by regulating the food and controlling the other conditions affecting the mother or nurse, to restore the milk to its normal composition, and thus remove the cause of disturbance. The practical importance of this can hardly be over-emphasized.

It is not necessary, for this purpose, to have always a complete analysis of the milk. According to Dr. L. Emmett Holt, of this city, who has studied the question very thoroughly, the information given by the specific gravity and the percentage of cream is sufficient to determine any serious changes. The specific gravity of normal breast milk varies usually from 1.028 to 1.033, averaging about 1.031; while the cream which rises in twenty-four hours, corresponding to the normal average of from 3 to 4½% of fat, should keep within the limits of from 6 to 9% or so.

The following table will help to explain the results of the examination. It will be noticed that the casein and the fat vary independently of each other:

Human milk.	Specific gravity.	Cream in 24 hours.	Proteids (calculated).
Normal average	1.031 (70° F.)	7.5 to 8.5%	1.5 to 2%
Healthy variations	1.028 to 1.029	9 to 12%	normal (rich milk)
" "	1.032 to 1.033	5 to 6%	" (fair milk)
Unhealthy "	below 1.028	high (above 9%)	normal, or slightly below
" "	" "	normal (6 to 9%)	low
" "	" "	low (below 6%)	very low (very poor milk)
" "	above 1.033	high	very high (excessively rich)
" "	" "	normal	high
" "	" "	low	nearly normal

The specimen for examination should be either the entire product of one breast, or taken after the breast has been about one-half emptied.

By using specially constructed hydrometers, and by determination of the cream in 10 c.c. graduated cylinders, the quantity of milk needed is reduced to a minimum.

KOUMYSS.

This is the general name given at present to any fermented alcoholic beverage made from milk.

History.—Its preparation and use have been introduced from the plains of southern Russia, where the various Tartar tribes, for many hundred years, have been accustomed to employ it, not only as a stimulant, but as a food.

Preparation.—Originally it was always prepared from mare's milk, but nowadays it is usually manufactured from cow's milk, skimmed and generally sweetened so as to resemble the other more closely. To produce the alcoholic fermentation in milk-sugar a peculiar ferment must be used, the kor or kefir ferment, the original of which came from the Steppes. This occurs as small irregular-shaped grains about the size of a pea, with a peculiar chocolate-like odor. It seems to contain, besides two forms of the ordinary yeast plant, a peculiar bacillus (Dispora caucasica), the whole cemented together with a sort of gummy material. It is supposed that by the action of the bacilli the lactose is converted into the glucose galactose, which then undergoes alcoholic fermentation in the ordinary manner.

Composition.—Besides the alcohol and carbon dioxide resulting from this, koumyss always contains some lactic acid from the action of some of the ordinary bacteria, and also traces of butyric and acetic acid. The casein is first coagulated by the acid, and then broken up into fine particles by the agitation which is a necessary part of the process of manufacture, while some of it seems to be actually decomposed into albumoses or similar bodies.

Uses.—Koumyss is very largely used at present as a food stuff, in cases of malnutrition or of wasting disease. It has a not unpleasant, sour, rather pungent taste, which to many people is extremely palatable; and besides acting as a slight stimulant, owing to the presence of carbon dioxide and small quantities of alcohol, it furnishes the constituents of milk in a very digestible form.

Analyses.—The following analyses of koumyss, given by König,* may be of interest:

KOUMYSS FROM MARE'S MILK, 43 ANALYSES.

	Alcohol.	Lactic Acid.	Nitrogenous Sugar.	Nitrogenous Matter.	Fat.	Ash.
Minimum,	0.15	0.34		1.12	0.80	0.28
Maximum,	3.29	2.92	6.80	3.73	2.56	0.77
Average,	1.91	0.91	1.77	2.24	1.46	0.42

KOUMYSS FROM COW'S MILK.

Average,	1.14	0.55	4.09	2.66	1.83	0.43

KOUMYSS FROM SKIMMED COW'S MILK.

Average,	1.38	0.82	3.95	2.89	0.88	0.53

LABORATORY EXPERIMENTS.

MILK AND KOUMYSS.

I. Cow's Milk.—1st. *Specific Gravity.*—Test some skim milk with the urinometer, and notice that it stands at about 1.033. Use this milk for the tests 2d, 3d, and 4th. During the lesson make comparative tests with the lactometer on whole, skimmed, and watered milk and on cream, noticing the reading on the scale, the appearance of the liquid on the bulb, and the way in which the liquid drips on lifting out the instrument.

Also place a drop of each under the microscope and observe the comparative abundance of the fat-globules.

2d. *Casein.*—Put some skim milk in a test-tube, add a little HNO_3 dil., and notice the coagulation of the casein. Warm the rest of the skim milk in a beaker on a water bath to 38° or 40° C., add a few drops of rennet, and let it stand quietly until it coagulates. Cut the curd with a knife, and decant or filter off the whey for Tests 3d and 4th. Test the casein: (*a*) With the xanthoproteic reaction. (*b*) With Millon's reagent. (*c*) Warm gently on the water bath with HCl conc. = violet colored solution.

* Loc. cit., pp. 418–419.

(*d*) Warm with water and a few drops of KOH = solution. Reppt the casein from this solution by neutralizing it with very dilute HCl, or better, dilute acetic acid.

3d. *Milk Sugar, Lactose.*—Test the whey for lactose: (*a*) By Moore's test. (*b*) By Trommer's test. (*c*) By Fehling's test. (*d*) By the picric-acid and potash test. For the details of these tests see Lesson II.

4th. *Inorganic Constituents.*—Put the rest of the whey in a small evaporating-dish, add a few crystals of $NaNO_3$, and evaporate it gently over a small flame, stirring it constantly. When it gets down to dryness, heat it cautiously until it ignites with more or less spattering, and, after that, heat it strongly until most of the organic matter is burnt white. Let it cool, add a little water and HNO_3 dil., warm gently, and filter. Test the solution in separate test-tubes:

(*a*) For *phosphates*—with excess of $(NH_4)_2MoO_4$.

(*b*) For *chlorides*—with a drop or two of $AgNO_3$.

(*c*) For *sulphates*—with a drop or two of $BaCl_2$.

The tests (*b*) and (*c*) will probably be very faint.

5th. *Butter Fat.*—Put the cream into a small evaporating-dish, add an equal amount of alcoholic potash solution, and heat over the water bath for some time. Notice the characteristic smell of butyric ether. Then add a few drops of dilute H_2SO_4, and notice that the same smell becomes more distinct.

Compare this with the butter tests in Lesson VII.

II. Clinical Tests on Breast Milk.—Whenever it is possible the student will be provided with a small sample of breast milk. It should be examined as follows:

(*a*) *Specific Gravity.*—Determine the specific gravity *accurately* by means of the small clinical hydrometer (to be obtained from the demonstrator).

(*b*) *Percentage of Cream.*—Stir the milk carefully and then fill up to the zero mark the 10 c.c. graduated cylinder on your desk. Let the milk stand for twenty-four hours; then examine it and note carefully the percentage of cream.

Calculate, from the figures thus obtained, the probable proportion of the constituents of the milk, according to the directions given on p. 182.

III. Koumyss.—Examine some koumyss as follows:

1st. *Carbonic-Acid Gas.*—Notice how, when the liquid is poured into a beaker, the escaping gas will extinguish a burning match.

2d. Taste it and smell it.

3d. *Reaction.*—Notice that it has an acid reaction to test paper, due to the presence of lactic, butyric, and perhaps acetic acids.

4th. *Casein.*—Heat the koumyss gently, in a beaker, till it begins to boil. Cool, separate the coagulated casein as much as possible from the whey, and filter enough of the latter to serve for Tests (*a*), (*b*), and (*c*). The tests for lactose can be made on unfiltered whey. Test the casein as above, under Milk.

If you have time test the filtered whey as follows:

(*a*) *Alcohol.*—Fill the flask not over one-quarter full, add a piece of pumice stone, and distil over very carefully into a test-tube, as in Lesson V.

Test the distillate for alcohol with the iodoform and with the molybdic-acid tests.

For the details of these tests see Lesson V.

(*b*) *Butyric Acid.*—Test the same distillate for butyric acid: 1st. By the smell. 2d. With test paper = acid. 3d. Add a few drops of common H_2SO_4 and shake = characteristic odor of butyric ether.

(*c*) *Dissolved Proteids.*—Test the filtered whey for these by the xantho-proteic and the biuret tests (*v.* Lesson VIII.).

(*d*) *Lactose.*—Test the whey for lactose, as above under Milk.

Microscopical Examination.—Examine carefully a drop of koumyss under the microscope. Notice the fat-globules floating on the top, the yeast cells lying on the bottom, and the innumerable bacteria floating all through the liquid. Notice also the comparatively large lumps of casein scattered through the mass.



LESSON XIX.

THE BLOOD.

Blood may be briefly described as a thick, viscid fluid, which circulates during life through all the tissues of the body. Its functions are varied and of the greatest importance, for it really serves as the sole medium of communication between the interior of the body and the outside world. It carries oxygen, and also nourishment of all sorts, to every part of the body, and removes all the waste products which would otherwise prove extremely injurious. It serves to keep the body temperature uniform, and to keep all the joints and tissues moist and in good condition; while some of its constituents are of great service in warding off the germs of disease, and in repairing and building up the tissues where lesions have occurred.

Besides this, the blood is the source from which all digestive fluids, and also all the secretions and excretions of the different glands and organs, are originally derived. It is interesting to notice how the same blood plasma is converted, by the action of cells, into such a range of fluids, with such different properties and functions, as the gastric and pancreatic juices, saliva, bile, milk, lymph, urine, and the many others which might be mentioned.

GENERAL PROPERTIES.

In man and in the higher animals blood is a red opaque fluid, owing both its color and its opacity to the enormous quantity of minute corpuscles suspended in it. During life we may consider it as composed of the thick, yellowish liquid known as plasma, and the blood cells, both red and colorless. When taken from the living blood-vessels, the blood coagulates, *i.e.*, some of its constituents are converted into the insoluble compound fibrin, which, when undisturbed, enfolds all the corpuscles within its

meshes, forming what is known as a clot and expressing a transparent, yellowish fluid, the serum. By manipulating a clot of blood, we can press out the blood cells into the serum and extract the fibrin by itself, thus producing defibrinated blood, a fluid very similar in most of its properties to the blood in the body.

Specific Gravity.—The specific gravity of human blood varies from about 1.040 to 1.075, the average being, for men, 1.058, and for women, 1.055. It varies more or less with age, and also with the amounts of food, drink, and exercise, the external temperature, and similar conditions. It is diminished, only temporarily, however, by hemorrhages.

Quantity.—The quantity of blood is usually about one-thirteenth of the body weight, and this, as well as the specific gravity, is kept within rather close limits by the kidneys, skin, and possibly the liver, spleen, and other organs.

Taste and Odor.—Blood has a rather insipid taste, due probably to the alkaline salts contained in it, and it has a distinct though faint odor which is quite characteristic of the animal from which it is derived. The odor is brought out much more distinctly by the addition of a little concentrated sulphuric acid, which sets free traces of volatile acids which have a more pronounced smell than the salts from which they are derived.

Reaction.—The reaction of blood is slightly alkaline, depending upon the presence of hydro-disodic-phosphate, and possibly of a little sodic carbonate. After shedding, the alkalinity diminishes, and on coagulation the blood becomes acid and increases in acidity as it stands.

It is a matter of some difficulty to estimate accurately the amount of this alkalinity. Careful experiments, however, by Von Jaksch and others, tend to show that it is often diminished in fever, and always in uræmia, liver disease, diabetes, and some other disorders. It is also diminished in rheumatism and in a gouty condition of the body, and one author, Cantani, insists that he has found it actually acid, during life, in the last stages of cholera. This, however, is exceedingly improbable.

Color.—The color of the blood is due to hæmoglobin, the coloring matter contained in the red corpuscles. The serum and plasma are both colored yellow or orange, and the individual

corpuscles, when seen under the microscope, have but a faint yellowish tinge which changes to red only when several of them are superposed.

In natural blood the hæmoglobin is contained in the blood cells only, and this makes the liquid thoroughly opaque. It is possible to extract the hæmoglobin from the corpuscles and cause it to dissolve in the serum, when the colorless body, known as the stroma, which forms the remainder of the blood cell, no longer interferes with the light, and we have a dark red, transparent solution. This is the case when blood is heated up to about 60° C., or is rapidly chilled and heated, or is subjected to electric sparks. The hæmoglobin can also be dissolved out by an excess of water, or by the admixture of chloroform, ether, alkalies, strong acids, and numerous other reagents.

On the other hand, the addition of solutions of neutral salts, such as NaCl, Na_2SO_4, and others, tends to shrivel up the blood cells, which retain firmly their coloring matter, and the result is a very opaque fluid with a bright vermilion color.

The difference in color between the arterial and venous blood is due to another cause, the presence of the hæmoglobin in the oxidized and in the reduced condition.

COMPOSITION.

According to several analyses by Sacharjin and Hoppe-Seyler,* healthy horse's blood contains, on an average, in 1,000 parts—

Blood corpuscles, 344.18 parts, of which
about 213 parts are solid material
and 131 " " water.

Plasma, 655.82 parts, of which
about 593 parts are solid material
and 62 " " water.

These figures would probably not vary much in the case of normal human blood.

With regard to the general composition of fresh human blood the following analyses are interesting. They were made by Becquerel and Rodier,† about 1844, upon specimens of blood from

* Hoppe-Seyler, "Physiol. Chem.," 1881, Part II.; p. 447.

† Quoted by Hoppe-Seyler, "Physiol. Chem.," p. 471; also by H. Vierordt, "Anatom. Physiol. u. Physikal. Daten u. Tabellen;" Jena, 1888.

eleven males and eight females, all adult and in fairly normal condition. The amount of hæmoglobin was calculated by Hoppe-Seyler from the percentage of iron.

	Males.	Females.
Water,	779.	791.
Solid materials,	221.	209.
The latter were composed of		
Fibrin,	2.2	2.2
Hæmoglobin,	134.4	121.7
Proteids,	76.	76.
Cholesterin, lecithin, and fats,	1.6	1.62
Extractives and salts,	6.8	7.4

Hæmoglobin.—This, the red coloring matter of the blood, may be considered as the most important of all the constituents in the above table, for it furnishes the means by which the blood carries oxygen from the lungs throughout the body.

Occurrence.—It occurs to a minute extent in the muscles of the higher animals, but it is practically all contained in the red blood cells of which, when dry, it forms, in man, from 80% to 90% by weight. The remainder of the corpuscle, known as the stroma, is a colorless, spongy mass, composed of albuminous matter with small amounts of cholesterin and lecithin. It is supposed to hold the hæmoglobin within its pores, perhaps in a state of loose chemical combination.

The coloring matters from the blood of different animals have the same general properties, but are sufficiently different in crystalline form, solubility, and chemical composition to show that they are not absolutely identical.

Preparation.—Hæmoglobin, in its oxidized condition, may be extracted from blood in several ways. From the blood of a rat or a guinea pig it readily crystallizes out, after a little standing, upon the simple addition of a few drops of water. When dealing with the blood of other animals, it is usually necessary to separate the red blood cells by centrifugal action or by settling, and then to decompose them with ether, letting the coloring matter slowly crystallize from the lakey solution.

Properties.—The oxy-hæmoglobin, extracted in this way, is in the form of dark red crystals, containing from 3 to 9% of water of crystallization. Most varieties of it dissolve but slightly in water,

and not at all in alcohol, ether and chloroform. It is somewhat soluble in dilute alkalies. The solutions are red by reflected, but green with transmitted light.

Composition.—The composition of the different varieties of hæmoglobin has been very carefully studied. According to some very careful analyses by Hoppe-Seyler,* the oxy-hæmoglobin from dog's blood is composed of—

C	H	N	O	S	Fe
53.85	7.32	16.17	21.84	0.39	0.43%

Of all the constituents mentioned above, iron is the characteristic one, giving the compound, it is believed, its power of absorbing oxygen. It is contained in human hæmoglobin to the extent of 0.42%, and thus furnishes an easy method for calculating the percentage of hæmoglobin in any sample of blood.

In this way we find that the average amount of hæmoglobin in human blood is from 12 to 15%, being somewhat less in women than in men. In cases of anæmia from various causes, this percentage of hæmoglobin may be reduced nearly one-half. Special pieces of apparatus have been devised both for counting the number of blood-corpuscles and for determining the percentage of hæmoglobin, more or less accurately, for clinical purposes. These have proved of much value, not only for diagnosis, but also for following the course of the disease.

Action of Oxygen.—The oxy-hæmoglobin just described is a loose chemical combination of oxygen with hæmoglobin itself. The latter can be obtained from aqueous solutions of the former by exhausting under a vacuum, or by reducing with a stream of hydrogen, or, more conveniently, with an alkaline solution of ferrous hydrate. In each of these cases the oxygen is extracted, leaving a solution of hæmoglobin. This same result takes place in the tissues, where the bright red arterial blood containing the oxy-hæmoglobin is reduced to the darker colored venous blood.

Pure or reduced hæmoglobin can also be crystallized, though with difficulty, and is somewhat less soluble than the oxidized form. It absorbs oxygen very readily, even on standing in the air, in the proportion of 1.16 c.c. of oxygen, at 0° C., and a pres-

* Hoppe-Seyler, "Medicin. Chem. Undersuch.," 1866-71; p. 370.

sure of one metre of Hg, for one gramme of the coloring matter.*

Methæmoglobin.—There is another compound of hæmoglobin with oxygen, called methæmoglobin, which is a more stable compound than the other, and has a brownish color when in solution. It is produced by slow oxidation, and hence is found in old blood stains, and sometimes in bloody urine, bloody cysts, and the like, where some time has elapsed since the effusion. It can also be prepared directly by oxidizing hæmoglobin with chlorate or permanganate of potash and other oxidizing agents.

Hæmatin.—When aqueous solutions of oxy-hæmoglobin are heated, especially in the presence of either acids or alkalies, it readily decomposes into a proteid of the globulin variety and about 4% of the red coloring matter hæmatin. Small quantities of carbon dioxide and volatile fatty acids are formed at the same time.

Hæmatin is a bluish-black, amorphous body, insoluble in water and alcohol, but soluble in dilute acids and alkalies. Its composition, according to Nencki and Sieber,† corresponds very closely to the formula $C_{32}H_{32}N_4FeO_4$. Its most interesting derivative is hæmin, a compound of hæmatin with two molecules of HCl.

Hæmin.—This substance is formed whenever nascent HCl is allowed to act upon a body containing either hæmoglobin or some of its derivatives. It forms extremely characteristic microscopic crystals, dark in color, and shaped like little rhombic plates or rods with sharp angular ends. When formed from minute quantities of blood they are very small, needing careful examination under a one-sixth objective. They are quite insoluble in water and dilute acids, but dissolve readily in alkalies.

Fibrin.—The formation and properties of this substance have been discussed in Chapter III.

The Serum.—This is the rather thick, transparent, yellowish or brownish fluid that is expressed from the clot when coagulated blood has stood quietly for some hours. It is blood after the removal of both fibrin and corpuscles.

It contains, all told, from 8 to 10% of total solids, of which 6 to

* Hüfner, Zeitsch. Phys. Chem., I., 1877-78; p. 388. † Berichte, xvii, '84; p. 2,270.

8% are proteids, and the rest are fat, salts, and minor constituents. Of the proteids the only two that have been so far isolated are serum albumin and paraglobulin, both of which have been described before. These are contained in varying proportions in the blood of different animals, human serum, according to Hammarsten,* containing on an average, 4.5% of albumin and 3.1% of paraglobulin, and bullock's serum, 3.3% of the first and 4.2% of the latter.

In the body we rarely find one without the other, and, as their significance is the same, the tests that we use for finding albumin in the urine and other fluids of the body are such as to include paraglobulin also. Still, it is possible to separate the latter from the serum albumin by saturating with CO_2, or with $MgSO_4$.

Special Tests for Albumin (and Paraglobulin).—The tests described are, on the whole, the most satisfactory when testing for small amounts of albumin. They will be mentioned again under Lesson XXIII.

The first of these tests, the addition of acetic acid and then boiling, is in very common use among physicians, and, while it works somewhat better in urine than in solutions of pure serum, it is on the whole eminently unsatisfactory. As will be noticed in this lesson, the slightest excess of acetic acid will spoil the test by forming the soluble acid albumin on heating. If, however, too little acid is added, the solution remains slightly alkaline, and the albumin does not precipitate, because it is changed into alkali albumin.

The next test, with acetic acid and potassic ferro-cyanide, in the cold, will be found more satisfactory. The modification where a ring is formed is particularly delicate.

The ring test with nitric acid, or Heller's test as it is often called, is, on the whole, the most satisfactory of them all. In our lessons the student is directed to use dilute nitric acid. The test is usually made with concentrated acid, and is then a little more delicate; but is more liable to error than in our method, because the strong acid may precipitate uric acid when used on a sample of urine. The test shows readily the presence of one part of albumin in 40,000 or 50,000 parts of water.

* Pflüger's Arch., 17; p. 459.

The ring test with picric acid is not quite as delicate as the last test, chiefly because the solutions are so nearly alike in density that it is hard to prevent them from mixing. The great advantage of this test, however, is that, after testing for the presence of albumin, the same solution, with the addition of some potash, serves also for determining the presence of glucose.

Paraglobulin.—This proteid has already been described in Lesson III. The separation of it from serum by means of CO_2 is not at all perfect unless the gas is allowed to pass through the liquid for some hours. Enough, however, for our test can be extracted in fifteen or twenty minutes.

Fats.—There is always a small amount of fat present in the blood, probably in a very finely emulsified condition. Its percentage varies greatly, accordingly to the food and condition of the individual. In the serum of fasting animals it is about 0.2%, while after a meal rich in fatty food it may run up as high as 1.25%. In the latter case the serum is often more or less milky. Besides the ordinary neutral fats, small quantities of cholesterin and lecithin and, perhaps, of fatty acids are also present.

LABORATORY EXPERIMENTS.

BLOOD.

I. **Odor.**—Put half an inch or so of blood in a small beaker and add a few drops of common H_2SO_4. Mix the two liquids and notice the peculiar odor of the blood.

II. **Hæmoglobin.**—Fill the bottle half full of a solution of defibrinated blood in 20 or 25 volumes of water. Then make up a "reducing solution" as follows: Put in a test-tube half an inch of a strong solution of $FeSO_4$, add the dry tartaric acid, and enough NH_4OH to make a clear dark green mixture. Add a little of this reducing solution to the blood in the bottle, and notice that the color changes to a *dark purple* = reduced hæmoglobin. Shake this up three or four times in the bottle with air, and notice that it becomes *red* = oxy-hæmoglobin. Reduce this again with the reducing solution, and again oxidize it by shaking.

Mix some blood in a beaker with 25 or 30 volumes of water, and notice that the blood dissolves, becoming "lake-colored," *i.e.* dark and transparent.

Mix some blood in another beaker with the same volume of 10% NaCl solution, and notice that the color changes to a bright vermilion and that the solution remains opaque. Examine under the microscope a drop of each of these last mixtures, and notice that in the first the red corpuscles are almost invisible, while in the second they are plainly seen, although much shrivelled and contorted.

III. **Fibrin.**—Notice the structure of fibrin, both dry and wet. Test the wet fibrin as follows:

1st. Xantho-proteic reaction.

2d. Millon's reaction, with the addition of a little water.

3d. Warm with some diluted KOH in a test-tube on a water bath = solution.

4th. Warm with some HCl conc. in a test-tube on a water bath = violet-colored solution.

5th. Warm with some acetic acid = a gelatinous mass.

IV. **Mixed Proteids.**—*General Tests.*—Fit a thermometer to a test-tube containing some undiluted serum. Heat very gently in a large beaker of water, and notice the temperature of coagulation, about 76° C.

Dilute some serum with four or five volumes of water. To some of this add one drop of very much diluted acetic acid, $HC_2H_3O_2$, and repeat the above test. (There will probably be an opalescence at about 63 to 66° C., and coagulation at about 80° C.)

Make the following tests on the diluted serum: 1st. Millon's test. 2d. Xantho-proteic test. Notice that the addition of HNO_3, even if dilute, to diluted serum will cause a ppt. 3d. Add alcohol = ppt. 4th. Add $HgCl_2$ = ppt.

Special Tests.—1st. *Acetic-Acid and Heat Test.*—Fill a test-tube nearly full of diluted serum, and to this add a drop or two of much diluted $HC_2H_3O_2$. Heat the top part of the mixture and notice the white ppt. or cloud. Notice that the least excess of acid spoils the test. Also notice that the resulting ppt. does not dissolve on the addition of some dilute HNO_3.

2d. *Acetic-Acid and Ferrocyanide of Potash Test.*—Fill a test-

tube half full of diluted serum, and to this add half an inch of $HC_2H_3O_2$, not diluted, and a few drops of potassic ferrocyanide, K_4FeCy_6. Notice the white, flocculent ppt.

Repeat this test as follows: Put in a small test-tube half an inch of a clear mixture of $HC_2H_3O_2$ with a quarter of its bulk of K_4FeCy_6. To this, down the side, add very gently, through a pipette, a little diluted serum. Notice the white ring where the liquids meet.

3d. *Nitric-Acid Ring Test.*—Put in a small test-tube half an inch of HNO_3 dil., and to this, down the side, add very gently, through a pipette, a little diluted serum. Notice the white ring where the liquids meet.

4th. *Picric-Acid Test.*—Put in a small test-tube half an inch of picric-acid solution, and to this, down the side, add very gently some diluted serum. Notice the white or yellowish ring, and, on mixing the liquids, the ppt.

N.B.—These last tests are those used in testing for albumin in urine. It is therefore very important to make them repeatedly with more and more dilute solutions of serum, and to compare carefully their respective accuracy and delicacy.

V. **Paraglobulin.**—Dilute some serum with eight or ten volumes of water, and pass through it, in a beaker, a stream of CO_2 gas, made by adding HCl dil. to some limestone in a flask with a delivering-tube. Notice the formation of a white turbidity = paraglobulin. Let it settle for a few minutes, filter off the white ppt., wash it with water, and dissolve it on the filter into a test-tube with a few drops of ½% NaCl solution. Test this solution carefully for paraglobulin with the biuret test.

VI. **Fats.**—Add half an inch of gasolene to a test-tube half full of serum, and shake vigorously. Let the gasolene rise to the top, and test it for dissolved fatty substances as follows:

1st. Put a drop on some warm water in a watch-glass. Let it stand a minute or two for the gasolene to escape, and then examine it, under the microscope if necessary, for globules of fat.

2d. Soak up the rest of the gasolene with a piece of filter paper. Let it dry, and notice the greasy appearance and touch of the paper.

salt, four or five small crystals being quite enough; he must use the strongest kind of acetic acid; he must be sure that the mixture fairly boils; and, most important of all, he must not mistake for hæmin crystals the large, colorless, more or less irregular crystals of sodium chloride, or sodium acetate, which are always present.

The hæmin crystals produced in this test vary somewhat, in size and appearance, according to the variety of blood and the exact proportion of the reagents used. They can be generally described, however, as extremely minute, dark colored, sharp-angled rods or prisms, see Fig. 8. They can only be seen by careful examination of the preparation with a lens of high power.

This test possesses two or three important advantages over the others described in this lesson. It can always be obtained, by careful manipulation, from any kind of blood stain, however old and however dried up. Besides this, there is no stain that we know of, excepting blood, which will produce similar crystals.

The chief, if not the only, disadvantage is that it is not always easy to exhibit these crystals, under the microscope, to an average jury. This objection, however, can be partly overcome by the aid of photography.

Examination for the Blood Corpuscles.—The hæmin test gives us no idea as to the source of the blood. The only chance of finding that out is to examine the stain under the microscope, in the hope of identifying the individual corpuscles.

This is a matter of very considerable difficulty. After only a few hours' drying, the corpuscles shrivel up and become indistinguishable, so that from the examination of a dry stain no results at all can be obtained. The only way to bring the corpuscles back to at all their original size and shape is to moisten them very carefully with glycerin, or with a 0.2% salt solution, or with some other liquid which has as nearly as possible the specific gravity of the original blood plasma. Even in this case it is very rare to find a corpuscle in at all a perfect condition. If they are moistened with water or with some other light liquid, they swell up very much and finally burst; while if the liquid is too heavy, they are shrivelled all out of shape.

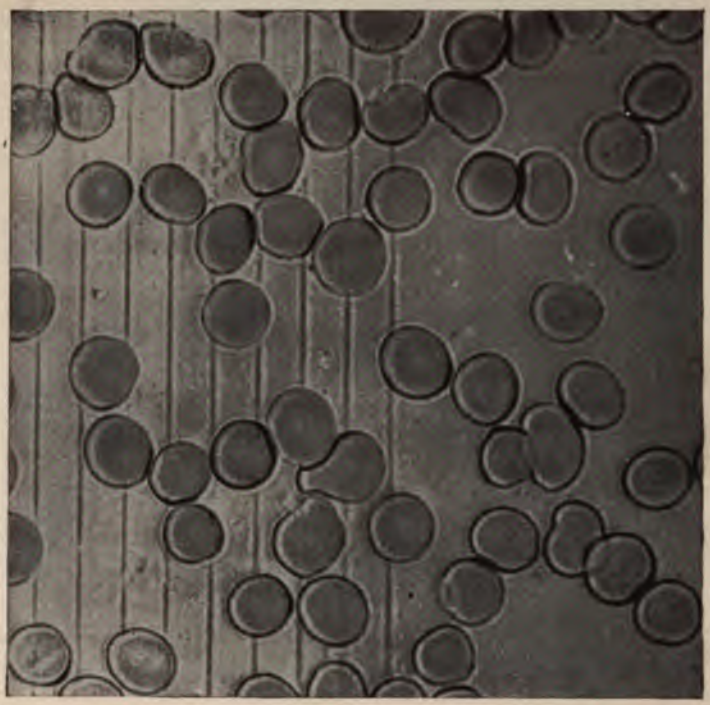


FIG. 1. Human Blood-Corpuscles, x 1150; average diameter = 0.000327 inch; lines = 1-5000 inch. From microphotograph by Dr. J. J. WOODWARD, U.S.A.



FIG. 2. Dog's Blood-Corpuscles, x 1150; average diameter = 0.000340 inch; lines = 1-5000 inch. From microphotograph by Dr. J. J. WOODWARD, U.S.A.

It is best not to look for the blood corpuscles in the middle of a great mass of blood, but to examine on the outskirts of a small stain, where only two or three corpuscles probably settled originally. This is especially the case when the stain is adhering to a fibrous material like cloth.

When, finally, a more or less perfect corpuscle has been found, it is necessary, in any medico-legal examination, to determine, with the utmost care, not only its exact shape, but also its exact size. In any important expert case the evidence of the eye is not sufficient. The corpuscle ought to be photographed in connection with the proper scale, so that the jury and any opposing experts can have an opportunity of studying it for themselves.

The shape of the human corpuscle is the same as that of all other mammalia excepting the camel family, *i.e.*, a flat round disk, with a depression in the centre of each side. This depression however, can hardly ever be distinguished in a case of this sort, the corpuscle in almost every case swelling to a globular mass.

The blood cells of birds, fish, and reptiles are oval, with distinct raised nuclei in the centre, and hence are readily identified. But the cells of many common mammalia not only have the same shape, but are so nearly of the same size as the human blood corpuscle that it is practically impossible to distinguish between them. The average diameter of the human cell is about 7.7μ * (0.0077 mm.), as against 7.3 for dog's, 6.5 for cat's, 5.9 for bullock's, 5.8 for horse's, and 5. for sheep's blood. Besides these common varieties, the blood cells of monkeys, guinea pigs, hares, mice, and a few other animals are very nearly of the same size as the human corpuscle.

But even these slight variations in size cannot be depended upon, for the individual cells of each variety have a very considerable range in size. This renders it quite hopeless to identify scattered cells, even of animals whose average cells differ widely. Thus, the human corpuscles vary in size all the way from 6.4 to 8.6, a range which covers individual cells from almost all the animals mentioned above.

Accordingly, while in medico-legal work it is often possible to positively identify a given sample of blood as mammalian blood, it is impossible to ascertain, by any test as yet known, whether

* Welker, Zeitschr. f. ration. Med. (3), xx., p. 279.

it was originally derived from man or from a dog, and very difficult to distinguish the blood of other common mammalia.

The Guaiacum Test.—This test is an interesting one, and reacts readily with minute quantities of blood, both fresh and old.

The reagents used are fresh solutions, 1st, of gum guaiacum, and, 2d, of hydrogen peroxide.

The gum guaiacum has the property of turning blue when in the presence of nascent oxygen, which, it is supposed, is set free from the hydrogen peroxide by the action of some of the ingredients of blood.

The test is an extremely delicate one, especially if the guaiacum solution is diluted with alcohol. But it has the serious disadvantage of being not so much a test for blood as a test for protoplasm in any form. In fact it works equally well with almost any cell contents, animal or vegetable. In our lesson we exhibit its action upon potatoes, and it will also react with milk, seminal fluid, and many other similar objects.

The chief value of this test is a negative one. A stain which does not react with guiacum in this manner cannot be blood.

The Spectroscope.—This instrument is often used for the detection of blood, and, in the hands of an experienced observer, it is extremely satisfactory. The different spectra, belonging to the "absorption" or "dark band" class, produced by hæmoglobin and its derivatives, are perfectly characteristic of blood, and blood only; while they can often be satisfactorily demonstrated, if necessary, to jury and experts.

The suspected material ought to be carefully isolated and soaked for some hours in a few drops of water, with frequent pressing and stirring. The liquid is clarified as much as possible by straining and filtering, any coloring matter left behind in this process being carefully washed back, and then is concentrated at a very gentle heat or, better, by standing over a desiccator, until it is ready to be examined under the spectroscope in a little cell. If the amount of material taken is very minute, the solution is often evaporated to dryness in the bottom of a watch glass and the examination made upon the little film remaining. The spectroscope should be arranged so as to throw, for comparison, a spectrum of real blood alongside of the spectrum of the suspected material.

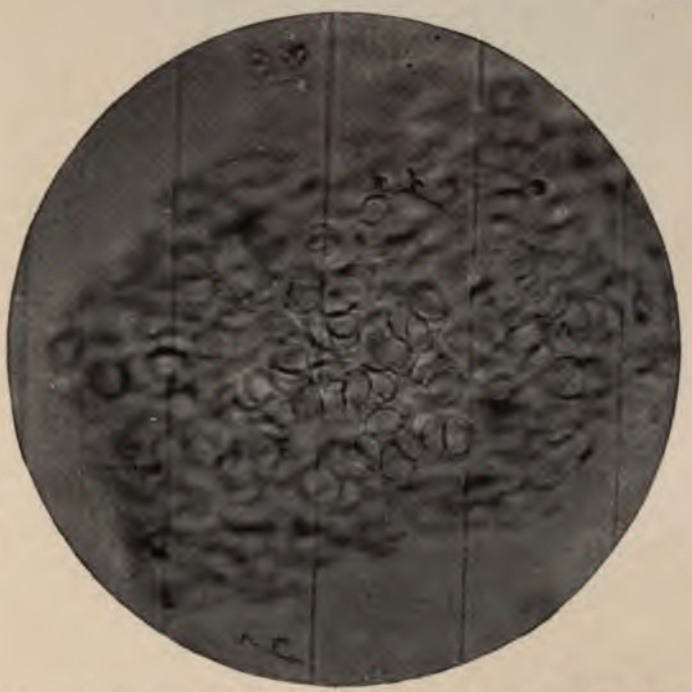


Fig. 1. Human Blood-Corpuscles, from dried stain soaked out, x 800; lines = 1-1679 inch. From microphotograph by Dr. J. J. Woodward, U.S.A.

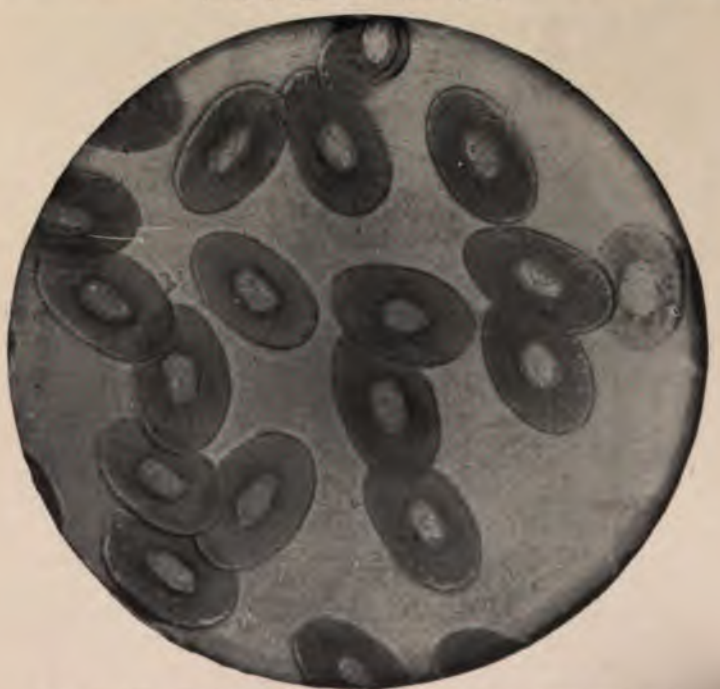


Fig. 2. Blood-Corpuscles of Frog. x 800. From microphotograph by J. J. Woodward, U.S.A.

If the stains are from fresh blood, the spectrum of oxyhæmoglobin may be expected. If, however, the blood has been standing for any length of time, the hæmoglobin present will probably have been oxidized to met-hæmoglobin.

The chief drawback to this method of testing is that it needs very careful manipulation, and also requires an expensive and somewhat delicate piece of apparatus.

THE BILE.

Occurrence.—The bile is a fluid, originating in the liver, which in part passes directly into the intestines, but most of which is first stored temporarily in the gall bladder, where it is mixed with more or less mucous secretion.

Preparation.—It can be occasionally obtained through fistulæ in living animals, but is usually taken from their gall bladders after death.

Properties.—(*a*) *Physical.*—Its character varies more or less with the species and with the individual from which it is obtained. In general, it is a rather thick, viscid fluid, somewhat stringy from the mucin added to it in the bladder. On shaking, it forms a thick froth or foam like the lather of soap. It has a faint, aromatic odor and a characteristic, intensely bitter taste.

Its color varies from yellow, orange, or brown, in the case of human bile and that from many carnivorous animals, to quite a pronounced green in the bile of many herbivora, such as sheep and bullocks. These colors, however, always intermingle more or less. When taken from the gall bladder, human bile has a specific gravity of 1.026 to 1.032 or so, but is somewhat lighter, about 1.010, when drawn directly from the liver in fistulæ.

(*b*) *Chemical.*—Bile has a neutral or faintly alkaline reaction. It dissolves readily in water, but when mixed with alcohol gives a precipitate of mucin. The bile acids, mixed with some coloring matter, can be precipitated by the addition of strong acids.

Its chemical composition varies considerably, the percentage of solid matter, in specimens of human bile taken from the bladder, ranging from 8 or 9% up to nearly 20%. This depends partly on the amount of mucous secretion that is mixed with it, and also, to a great extent, upon the length of time that it stands in

the gall bladder, which is constantly absorbing water from the bile contained in it. The proportion of the principal constituents, according to analyses made by Hoppe-Seyler* and others, seems to be about as follows:

Cholesterin,	0.2 to 0.4%	Bile pigments and mucin,	1 to 3%
Lecithin,	0.2 to 0.5%	Bile salts,	6 to 10%
Soaps and fats,	1.5 to 2.5%	Mineral salts,	0.6 to 1%

These substances will be discussed later.

(*c*) *Physiological.*—So far as we can tell, some of the constituents of the bile, the pigments for example, are purely waste matters; others, like the bile salts, are excreted from the liver, but reabsorbed in the intestines; and still others, little understood at present, are of considerable value in assisting the processes of digestion.

Functions.—The presence of bile is essential for the proper digestion of the fats. It seems to assist in their emulsification and to aid their passage through the intestinal walls.

Bile keeps the intestines and their contents moist and smooth, and thereby materially assists the passage of food along the alimentary canal. Hence many valuable purgatives, calomel for example, owe their effects to an increase in the flow of bile. It also decidedly increases the peristaltic action, and is often administered in pills and in enemata for that purpose.

Bile is supposed to have some antiseptic action upon the contents of the intestine, and it is a fact that in the absence of bile the fæces are unusually offensive and show marked signs of decomposition. Its value in this respect, however, may be due to its alterative and purgative action rather than to any specific effect upon the germs, for bile, outside the body, readily decomposes and putrefies.

CONSTITUENTS OF BILE.

CHOLESTERIN.—$C_{26}H_{43}OH$.

Occurrence.—This substance is found in small quantities in the bile of all animals, where it is probably held in solution by the bile salts. Under certain little understood but more or less dis-

* Hoppe-Seyler, "Phys. Chem.," pp. 299 to 301.

eased conditions, it often precipitates from the bile while it stands in the gall bladder, forming, in connection with more or less mucus, what are known as gall stones. The latter vary in size from the head of a pin to lumps the size of a pea or larger, and their passage from the bladder through the gall duct is frequently attended with most distressing, and even dangerous, symptoms.

Besides this, small quantities of cholesterin occur normally in the red and white blood cells, nerve tissue, brain, spleen, the intestinal contents, and in the fæces. It occurs also, under pathological conditions, in pus, tumors, tubercular deposits, cataracts, and in hydrocele and other pathological fluids. Cholesterin has been occasionally found in some samples of urine.

Preparation.—Cholesterin is best prepared from finely powdered gall stones, by thoroughly extracting them with boiling alcohol. The alcoholic solution is filtered off and allowed to evaporate slowly, and the cholesterin which crystallizes out can be purified by boiling with alcoholic potash or by resolution and recrystallization.

Properties.—(*a*) *Physical.*—Pure cholesterin crystallizes from solutions in benzol, chloroform, or anhydrous ether, in the form of fine, silky needles. From alcoholic solutions it separates in the form of large, flat, transparent rhombic plates, see Fig. 9, of the composition $C_{26}H_{43}OH + H_2O$. These crystals, after standing in dry air, lose a molecule of water and become opaque. They have a soft, greasy feeling.

Cholesterin is insoluble in water, cold alcohol, and concentrated alkalies. It dissolves readily, however, in hot alcohol, ether, chloroform, volatile and fatty oils, and in solutions of the bile salts. Its solutions polarize to the left.

(*b*) *Chemical.*—In composition it is a true monatomic alcohol, producing an acid on oxidization, and compound ethers on prolonged boiling with acids. Concentrated sulphuric acid melts its crystals down into a reddish mass, which, when the acid is mixed with a little iodine, is changed to blue.

(*c*) *Physiological.*—The functions of cholesterin in the animal economy are not known, but it is usually considered to be a pure excretion.

LECITHIN.—$C_{44}H_{90}NPO_9$.

Occurrence.—This substance, which, as well as cholesterin, has been already referred to more than once, occurs in bile to a small extent. It is found in quite large amounts in brain tissue, especially the gray substance, in nerves, blood cells, yolk of eggs, serum, milk, and other animal fluids and tissues. It also occurs very generally in seeds, stems, and other organs of plants, and, in fact, some physiologists claim that it exists in all cell contents, both animal and vegetable.

Properties.—(*a*) *Physical.*—It is a colorless, waxy, or slightly crystalline substance, very hygroscopic, and swelling in water to a soft pasty mass. It dissolves readily in chloroform and benzol, and also in hot alcohol and ether.

(*b*) *Chemical.*—In composition it may be considered as a compound of neurin or cholin, $N.(CH_3)_3.C_2H_4OH.H$, with distearylglycerin phosphoric acid, $C_3H_5.(C_{18}H_{35}O_2)_2.H_2PO_4$. This latter may be considered as ordinary stearin, $C_3H_5.(C_{18}H_{35}O_2)_3$, with one stearyl group replaced by phosphoric acid. Hence, while lecithin contains both nitrogen and phosphorus, it still, in composition as well as in other properties, much resembles a true fat. Very possibly instead of one there may be a whole series of lecithins, derived from olein, palmitin, and other fats in the place of stearin.

(*c*) *Physiological.*—The part that is played by lecithin in the nourishment of cells is not at present understood. Perhaps it is an intermediate stage in the formation of the fats.

BILE PIGMENTS.

These bodies consist mainly of the two coloring matters, bilirubin (yellow or brown) and biliverdin (green), with much smaller quantities of derived products, such as biliprasin, bilifuscin, and others.

They occur regularly in the bile. In disease of the liver they are often found in the urine, and also, in minute quantities, in many of the tissues and fluids of the body.

Bilirubin.—$C_{32}H_{36}N_4O_6$. This pigment is found to quite a large extent, either free or combined with some alkali, in the yellow or brown colored bile of man and of the carnivorous animals.

It can be extracted from the red gall stones, which are mostly a compound of bilirubin and calcium, by first treating them with hydrochloric acid, and then dissolving the liberated coloring matter with chloroform.

It forms transparent, red, rhombic crystals, which are quite insoluble in water, and almost so in ether and alcohol, but which dissolve readily in chloroform, especially on warming. This latter property distinguishes it from biliverdin.

It acts like a monobasic acid, forming soluble compounds with the alkaline metals, and insoluble salts with calcium, barium, silver, and lead. It is probably identical with a crystalline substance known as hæmatoidin, which is formed from the decomposition of hæmoglobin, and is occasionally found in the body in cases where blood has escaped into surrounding tissues and formed thrombi or plugs. This makes us believe that the coloring matters of the bile, which give the color to the fæces and probably to the urine also, are derived directly from the breaking down, in the liver, of red blood cells.

Biliverdin.—$C_{16}H_{18}N_2O_4$. This is derived from bilirubin by oxidation. It is found principally in the bile of the herbivora. It is also found in the walls of the placenta of certain animals, and can be extracted from these in a pure form.

It is an amorphous, dull green substance, insoluble in water, ether, and chloroform, and readibly soluble in alcohol. It is dissolved by alkaline solutions, even if very dilute, and is precipitated by acids or by solutions of lime, barium, and lead.

Tests.—*Gmelin's Reaction.*—The best test for these bile pigments is the formation of a series of highly colored substances, by the addition of a strong oxidizing agent like concentrated nitric acid. The latter acts best if it is yellow, *i.e.*, if it has some of the other oxides of nitrogen, NO_2 and N_2O_3, dissolved in it.

The color that first results is green from biliverdin. Then come, with successive oxidization, blue from bilicyanin, violet and red, from biliprasin, and finally yellow or even brown from a more highly oxidized body, choletelin, $C_{16}H_{18}N_2O_6$. This test can be made in different ways, although the general result is the same.

The other two tests, Ultzmann's test and the iodine test, de-

pend upon the same principle. The bilirubin is oxidized to biliverdin, in the first case by the air, on shaking with an alkali, and in the other case by the action of iodine. The reactions are interesting, but not so delicate as the Gmelin's test.

MUCIN.

Occurrence.—This is a nitrogenous substance belonging to the albuminoid compounds mentioned on page 73. It is the principal secretion from the mucous glands, which are scattered over the moist epithelial surfaces all over the body. Hence we find mucin in fluid which bathes the inner surfaces of the nose, mouth, throat, œsophagus, the whole alimentary canal, the bladder and gall bladder, and similar surfaces.

Properties.—It swells up in water, especially in the presence of solutions of neutral salts, and forms a slimy mass which is met with in bile and also, to a less extent, in urine. It is readily recognized in the secretion from any inflamed mucous membrane. It is precipitated by acetic acid, but is dissolved by alkalies, and can be changed, by continued warming with acids and alkalies, into acid and alkali albumins respectively.

Its composition varies somewhat with its source. Under some circumstances it responds to the glucose reactions, and, it is claimed, can be decomposed into glucose and a proteid body like acid albumin.

It is of great importance in serving as a lubricant.

THE BILE SALTS.

These compounds, which are peculiar to bile and which give it its characteristic bitter taste, are the sodium salts of glycocholic and taurocholic acids. In herbivorous animals the glycocholates predominate, while they are almost wholly absent in the bile of many carnivora.

The acids themselves can be readily obtained from bile by the addition of strong mineral acids. They form colorless crystals and dissolve readily in alcohol and chloroform, and less readily in water. Glycocholic acid, indeed, hardly dissolves at all in cold water, although soluble on heating. The acids and their salts all polarize to the right.

Both of these acids decompose readily, by boiling with acid or alkali, or by the action of organized ferments, into cholic acid and the substances glycin and taurin respectively.

The reactions are as follows:

$$\underset{\text{Glycocholic Acid}}{C_{26}H_{43}NO_6} + H_2O = \underset{\substack{\text{Glycin or}\\ \text{Amido-acetic Acid}}}{C_2H_5 . NH_2 . COOH} + \underset{\text{Cholic Acid}}{C_{24}H_{40}O_5}$$

$$\underset{\text{Taurocholic acid}}{C_{26}H_{45}NSO_7} + H_2O = \underset{\substack{\text{Taurin or}\\ \text{Amido-ethyl-sulphuric Acid}}}{C_2H_4 . NH_2 . HSO_3} + C_{24}H_{40}O_5$$

Cholic acid, which occurs sometimes in stale urine in cases of liver disease, is a monobasic acid, forming readily soluble, crystalline salts with the alkaline metals.

Pettenkofer's Test.—Solutions of the bile acids or of their salts exhibit a brilliant cherry-red color, changing, on standing, to a deep purple, when treated with concentrated sulphuric acid after the addition of a few drops of a 25% solution of cane sugar. The temperature of the mixtures should be kept as low as possible and an excess of sugar should be avoided, on account of the danger of producing caramel.

A similar color is produced by the action of sulphuric acid, either by itself or in the presence of sugar or strong acetic acid, upon proteids (compare the seventh test on p. 82), and also upon cholesterin, lecithin, many oils and fats, morphine, codeine, salicylic acid, and several other organic bodies. In order to identify the presence of bile acids by this test, it is necessary to evaporate the suspected fluid to dryness, extract it with absolute alcohol, and precipitate any bile acids that may be present, from the alcoholic solution, by an excess of ether. This precipitate, when dissolved in a little water, may then be tested with sulphuric acid and sugar.

GLYCOGEN.

This substance has already been described in Lesson II.

LABORATORY EXPERIMENTS.

BLOOD (CONTINUED) AND BILE.

I. Inorganic Constituents of Blood.—Dissolve some dried blood in a beaker or small evaporating-dish with a little hot HNO_3 conc. Dilute with water, filter, and test the filtrate as follows:

1st. *Iron.*—Test for Fe (ic) with NH_4CNS.

2d. *Phosphates.*—Test for these by adding a few drops of the solution to a test-tube nearly full of $(NH_4)_2MoO_4$.

3d. *Chlorides.*—To the rest of the solution add a drop or two of $AgNO_3$ = white cloudiness of AgCl.

II. Blood Stains.—Test stains on cloth, both old and recent, as follows:

1st. *Hæmin Test.*—Cut or pull off one or two shreds of the cloth and mix them on a slide with four or five crystals of NaCl. Lay on this a cover-glass, and under it run a drop or two of *glacial* acetic acid. Heat gently over a low flame till it just boils. Let it cool for a minute or two, add a drop or two of water to replace the liquid that has evaporated, and examine under the microscope, with the high power, for crystals of hæmin.



FIG. 8.—HÆMIN CRYSTALS. × 250.

Repeat this test two or three times until you have no trouble in getting the crystals.

2d. *Blood-Corpuscles.*—Place two or three shreds on a slide and moisten them with a drop or two of ¼% NaCl solution. After a minute or two, examine them under the microscope (high power) for blood-corpuscles. Notice that in the old stains these will be almost obliterated, and that they are extremely indistinct, even in the fresh stains.

3d. *Guaiacum Test.*—Moisten the remainder of the cloth with water, and press it on a piece of filter paper = a reddish stain. Moisten this stain with a drop of tincture of guaiacum, and then add a drop of hydrogen peroxide (H_2O_2) = *blue*.

Cut a potato in two, and on the fresh section spread a drop of the guaiacum solution. Let it stand a minute, and notice that it shows signs of turning blue. Add a drop of H_2O_2, and notice that parts of it, at least, turn blue at once. The potato responds to this test more rapidly if it has been cut through a partly decayed portion, or through an "eye."

BILE.

I. *Cholesterin.*—Put the powdered gall stone into a small test-tube, add one inch of alcohol, and boil in a water-bath for four or five minutes. Let the residues settle for a minute or two, and then decant off the clear liquid into two small clean watch glasses. Warm these, very gently, for a minute or two over the water bath, and then let them stand quietly, without stirring, until the alcohol has evaporated. Then examine each carefully under the microscope (low power), noticing the characteristic, flat, transparent crystals of cholesterin. To one of these specimens, while still under the microscope, add with great care one drop of common H_2SO_4. Notice that the crystals melt down and turn *red*. To the other, also under the microscope, add very carefully a drop of a mixture of equal parts of common H_2SO_4 and of iodine solution. Notice that this reagent turns the crystals *blue*.

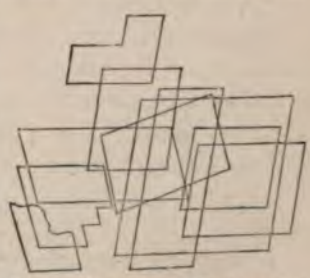


Fig. 9.—Crystals of Cholesterin. (Frey.)

N.B. Take great pains not to let acid fall on either lens or stand.

II. **Bile Pigments.**

(*a*) *Gmelin's Test.*—1st. Put in a test-tube half an inch of HNO_3 conc., and to this, down the side, add very gently with a pipette a few drops of bile. Notice the resulting colors.

2d. Put on a piece of filter paper a drop of bile, and on the

spot, when it has spread out a little, place a drop of HNO_3 conc. Notice the colored rings which result.

3d. Mix some diluted bile with a little HNO_3 dil. in a test-tube. Add gently down the side some common H_2SO_4. Notice the play of colors at the junction of the liquids.

(*b*) *Ultzmann's Test.*—Fill a small test-tube about one-third full of diluted bile, and add to it about half as much of a saturated solution of KOH. Shake it well, add an excess of HCl conc., and notice the emerald-green color which results.

(*c*) *Iodine Test.*—Put some diluted bile in a test-tube and add a few drops of iodine solution. Notice the green color which results.

Repeat the above tests with more and more dilute solutions of bile, and see which you consider the most delicate reaction.

III. **Bile Acids.**

Pettenkofer's Test.—Add very gently to some diluted bile in a beaker about two-thirds its volume of common H_2SO_4, mixing it carefully, and never letting the temperature rise above 60° C. Then mix in three or four drops of a 20% solution of cane sugar. Notice that the liquid first turns red and then changes to a deep violet color. Notice that this test, if carefully made, is quite delicate.

Dissolve some egg albumin in some common H_2SO_4 and add gently three or four drops of the same sugar solution. Warm very carefully to just 60° C., and keep it at that temperature for a few minutes. Notice that the liquid turns a violet color.

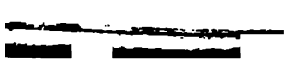
IV. **Glycogen.**— Dissolve the glycogen in hot water. Notice that it dissolves readily, but forms an opalescent solution. Test this solution as follows.

1st. Add a drop or two of it to a test-tube nearly full of alcohol. Notice that it ppts.

2d. To some of it in a test-tube add a drop or two of much diluted iodine solution. Notice that it is colored red or brown.

3d. Warm some of it in a test-tube with a drop or two of KOH. Notice that the solution becomes clear.

4th. Warm some of it in a test-tube for a few minutes with a drop or two of acetic acid. Notice that the solution becomes clear. Test it for glucose with Fehling's solution.



PART VII.

THE DIGESTION.



LESSON XXI.

DIGESTION.

THE food which enters the alimentary canal may be considered as passing along a tube lined throughout with a continuous membrane. Before the food can be distributed throughout the body, and be of service to the individual, it must first pass through this lining membrane, and then enter the blood or the lymph by traversing the walls of the capillaries or of the lymph-vessels, in the villi or other absorbing portions of the alimentary canal.

As is well known, the walls of the minute blood and lymph vessels are comparatively pervious to the blood plasma and hence to similar liquids. But it is a matter of much greater difficulty for the food to pass through the intestinal membrane. The latter is composed of a continuous layer of highly developed epithelial cells, backed up with cement substance. The function of the epithelial cells is not known, but it is generally agreed that the absorption of the fat, in the form of minute globules, is directly due to their agency or, possibly, to that of the leucocytes (white blood-cells), which, during digestion, are present in great numbers along the intestinal walls. While, however, the cells of the lining must have some modifying influence upon both proteids and carbohydrates, the absorption of the latter substances in the intestine seems primarily due to the physical process of diffusion.

DIFFUSION OF FLUIDS.

The phenomena of the diffusion of gases, *i.e.*, the scattering of their molecules throughout a vacuum or a space filled with other gases, have long been studied, and it has been satisfactorily proven that the rapidity of diffusion of a gas is inversely as the square root of its density. In other words, a given volume of hydrogen, whose specific gravity is 1, will pass through a small

opening or mix with a small amount of air or other gas four times as fast as the same volume of oxygen, whose density is 16. This is usually explained by stating that the "vis viva," or inherent activity, of a light molecule is naturally greater than that of a heavy one.

Colloids and Crystalloids.—It has been found in like manner that different liquids, or solutions of different substances, vary greatly in diffusive power, some, of their own accord, mixing rapidly, and others slowly. The speed of their diffusion seems to depend not so much upon the weight as upon the complexity and structure of the molecule. Hydrochloric, sulphuric, and many other mineral and organic acids diffuse very rapidly indeed, and alcohol, as well as solutions of common salt, sulphate of magnesia, sugar, and most of the ordinary salts and crystalline substances have quite a high diffusive power.

On the other hand, some mineral substances, such as silicic acid and hydrate of alumina, and especially certain organic bodies like starch, dextrin, caramel, albumin, gelatin, and the like, diffuse very slowly. These latter substances are, in almost every case, devoid of crystalline structure, and were named by Graham, who first investigated the subject, "colloids," from κόλλη (glue), as opposed to the diffusible compounds which he called "crystalloids." It must not, however, be forgotten that among the crystalloids or diffusible substances must be included a few amorphous compounds, such as the hydrates of soda and potash, and especially the peptones, while hæmoglobin and some of the crystalline vegetable proteids are striking examples of crystalline bodies which do not diffuse.

Dialysis.—Although colloid substances do not diffuse, they allow crystalloids to diffuse through them with almost as much readiness as through water. Thus, if a crystal of potassic chromate is placed on a mass of solid gelatin or of coagulated proteid, the whole of the colloid material will be colored yellow in a very short time. On the other hand, even small, thin layers of colloids are extremely impervious to colloid solutions. Thus, a piece of paper dipped in starch paste or a thin film of gelatin, placed so as to separate one liquid from another, will allow common salt or other crystalloid substances to pass through it with ease,

but will quite prevent the passage of albumen, or starch, or caramel. Instead of papers dipped in starch paste or a film of gelatin, it is more convenient to use either parchment paper (see pp. 7 and 120), when the sticky, tough surface acts as a colloid, or else animal membranes such as the linings of the bladder, intestines, œsophagus, and the like. It does not matter how fine the latter are; if the membrane is intact, and there are no holes in it, crystalloid substances will pass through and the colloids will stay outside. The separation of substances by this means is called *dialysis*.

Living animal membranes act in the same way as dead ones or as parchment paper. It is found that the rapidity of diffusion is increased by many conditions, such as warmth, motion of the liquid and of the dialyzing surface, difference in composition of the two liquids separated by the dialyzer, the removal of the crystalloids as fast as they diffuse through, and an increase in the dialyzing surface. But even if all these conditions are present, as they are, for instance, to a marked extent in the intestines, the thin animal membrane will still act as a barrier to the passage of any but crystalloid substances.

It so happens that almost all the ordinary food stuffs, starch, dextrin, the proteids, gelatin, and the like, are distinctly colloid bodies. Hence, when they reach the intestine, although separated from the blood and lymph vessels of the villi by only a very thin membrane, they cannot enter the system by a simple process of diffusion, unless altered in molecular composition.

Before, however, they reach the end of the absorbing part of the intestines, they have been thoroughly subjected to the action of the various digestive fluids, the saliva, the gastric and pancreatic juices, and, to a less extent, the intestinal juice and the bile. Under their influence the colloids, like starch and the proteids, are converted into crystalloid substances, maltose, glucose and peptones, which can pass readily through the intestinal wall and enter into the general circulation.

It will be noticed that in each case the new product belongs to the same class of proximate principles as the original food stuff, and also that there is but little loss of potential energy in the process, the digested food containing but little more oxygen

than it did before. We shall now discuss these digestive fluids more at length.

THE SALIVA.

This fluid is derived mainly from the salivary glands, but is always mixed with more or less secretion from the mucous glands of the mouth.

Properties.—It is a rather viscid, tasteless fluid, slightly turbid, and with a specific gravity ranging from 1.002 to 1.006. It has, in health, a slight alkaline reaction, averaging, according to Chittenden,* 0.08% Na_2CO_3, but in dyspepsia or where the secretion is very scanty it is sometimes neutral or even acid.

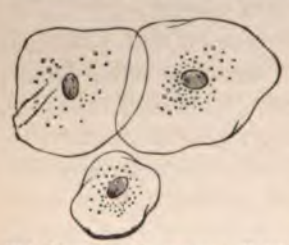


FIG. 10.—OLD EPITHELIAL CELLS FROM THE HUMAN MOUTH. (FREY.)

Composition. — When examined under the microscope, it is seen to contain epithelial and other cells from the mucous membranes of the mouth, the so-called salivary corpuscles, *i.e.*, round granular cells from the salivary glands, and great varieties of bacteria, and sometimes of other organisms. Of the bacteria, the most striking is the one called Leptothrix buccalis, which forms long, branching chains and is supposed to take part in the decay of the teeth. It can be best observed in a little tartar from the teeth, spread out on a slide with a drop of water or saliva.

Besides these, the saliva always contains more or less débris of food, and, in diseased conditions, such as inflammation of the nose or throat, there are often present both pus cells and broken-down epithelia.

It contains, in solution, about 0.5% of solid matter. Of this, rather less than one-half is mineral, composed, as in the case of the blood and milk, of the chlorides, phosphates, and possibly carbonates of sodium, potassium, calcium, and magnesium. It contains in most cases small quantities of potassic sulpho-cyanide, KSCN, a substance not, so far as we can tell, present in the blood, nor of any special importance, but which can be detected

* "Studies from the Laboratory of Physiol. Chem., Yale College," vol. i., 1884–85.

by the red color it produces in a very dilute solution of ferric chloride.

Among the organic constituents, we find mucin, which gives it its consistency, traces of proteid bodies, chiefly albumin and globulins, and the ferment ptyalin.

Ptyalin.—This substance is found in the saliva of man and of several of the higher animals. It can be precipitated from saliva by an excess of alcohol, and, when separated and dried, it forms a white powder very soluble in water. It decomposes readily in an aqueous solution.

As before explained (see Lesson III.), it has the property of converting hydrated starch into dextrin and maltose, and finally into dextrose. Its action is much weaker than that of either the diastase of malt or the amylopsin of the pancreatic juice. It works best, according to Chittenden, in neutral solutions, although it still acts in solutions containing as much as 0.2 to 0.3% Na_2CO_3. The addition of more alkali retards and soon destroys it. It is very quickly injured by even faint traces of acids, if they are free. Thus, free hydrochloric acid of a strength of only 0.003% stops the action, and of 0.005% kills it. The presence of proteids protects the ferment for a while from the action of the acid by forming acid albumins with the latter. But even in the stomach, where the acid of the gastric juice is weak and the albuminous material in the food supply often quite large, it is doubtful if the ptyalin survives more than a few minutes.

Uses.—Although saliva contains the ferment in appreciable quantities, it is not believed to have much value as a true digestive fluid. The saliva, however, is of the utmost importance, not only in keeping the mouth and œsophagus moist and smooth, but also in lubricating the food and putting it into a condition in which it can pass easily and rapidly into the stomach.

GASTRIC JUICE.

The food, more or less disintegrated by the teeth, and with some of the starch changed to sugar, passes into the stomach to undergo the second stage of digestion.

Preparation.—The gastric juice can be readily obtained, in a fairly normal condition, not only from dogs and similar animals,

but also from man, by the aid either of gastric fistulæ or of the stomach pump.

Properties.—It is a thin, almost colorless liquid, with a somewhat sour taste. Its specific gravity varies from 1.001 to about 1.010, and it contains only small amounts (in man, probably from ½ to 1%) of solid matter.

Its reaction is distinctly acid from the presence of free hydrochloric acid to the extent of from 0.1 to 0.3 or even 0.4%. Some of the organic acids, principally lactic and butyric, are frequently present in the stomach, sometimes in comparatively large amounts. It is probable, however, that these are not secreted in the gastric juice itself, but produced by some fermentative action from the food after it has entered the stomach. Its acidity is also sometimes affected by the presence of acid salts, generally the acid phosphate of soda, NaH_2PO_4.

Composition.—Of the solid matter present in the gastric juice, nearly one-half is composed of mineral matter, principally the phosphates and chlorides of the alkaline and earthy metals. The organic matter consists chiefly of the unorganized ferment pepsin and of a little mucin, already described. There is also present, especially in the stomachs of infant mammalia, some of the rennet or milk-curdling ferment, mentioned in Lesson XVIII.

Pepsin.—Pepsin can be prepared in a more or less pure form by two or three different methods, such as, for instance, by extracting the mucous membrane of the cardiac end of a pig's stomach with glycerin and then precipitating the ferment with strong alcohol. Prepared in this or in other ways it is a grayish-white, amorphous powder, which is not hygroscopic, and dissolves slowly in water, and more readily in dilute acid. Its solutions can be precipitated by plumbic acetate or by platinic chloride, but not by mercuric chloride, concentrated nitric acid, or even by tannin. It does not diffuse through parchment paper or animal membranes.

Action.—Pepsin, when mixed with small quantities of hydrochloric or, to a less extent, of other acids, has the property, under favorable conditions, of breaking down proteids and many albuminoids into albumoses, and finally into peptones. These bodies are products of hydration, containing more oxygen and hydrogen

and less carbon than the original proteids; and they can be formed by the action of the pancreatic ferment trypsin in an alkaline solution, or by hot sulphuric acid, as well as by gastric juice.

Both the peptones, and the albumoses from which they are derived, are divided into two distinct classes, known by the prefixes "hemi" and "anti." The hemi-peptones, when subjected for a long period to the action of trypsin, are broken down into the compounds leucin, tyrosin, and small quantities of naphthylamin. The anti-peptones, on the other hand, resist the action of the pancreatic juice, and cannot be decomposed by it.

Albumoses.—The albumoses, of which some four varieties have been distinguished, are all soluble in dilute sodium chloride solutions, and are precipitated, either wholly or in part, by an excess of that salt. They resemble the ordinary proteids by giving a violet color with the biuret test, and by being precipitated by acetic acid and potassic ferrocyanide. They can be wholly precipitated by saturating their solution with crystals of ammonic sulphate. They are not diffusible.

Peptones.—The peptones represent the final product of peptic digestion. They are very hygroscopic, and are exceedingly soluble in water. They are precipitated by only a few reagents, tannin, alcohol, mercuric iodide dissolved in potassic iodide, and, to some extent, by solutions of either picric, phospho-tungstic, or phospho-molybdic acids. They are not precipitated by ammonic sulphate, and differ from the other albuminous substances by giving a rose-color with the biuret test, and by being readily diffusible.

These peptones, although the final products of the action of gastric juice, are probably actually formed to but a small extent in the stomach itself. They are produced in great abundance by the action of the pancreatic juice upon the partially digested food, and represent the form in which the proteids pass through the wall of the intestines. They are found, however, in such minute amounts in the blood of the portal vein that it is believed that, by the action of the cells of the intestinal membrane, they are reconverted into proteids, such as serum albumin, paraglobulin, and fibrinogen, during the process of absorption.

Influences Modifying the Action of Pepsin.—The hydrolytic action of pepsin depends directly upon the prior conversion of the proteid into acid albumin or syntonin. This is best done by dilute hydrochloric acid, but will also take place in the presence of phosphoric, sulphuric, or oxalic acid. A certain amount of free acid is also necessary for the action of the pepsin itself. When nitric acid is substituted for hydrochloric acid the pepsin works with much less energy, and with acids like acetic, lactic, and butyric its action is either greatly diminished or entirely stopped. Hence, in cases of dyspepsia the presence of a strongly acid gastric juice, due to even large quantities of the valueless organic acids, is not a counter-indication to the giving of small doses of free hydrochloric acid.

The action of pepsin is decidedly stimulated by the presence of small quantities of sodium chloride and, to a marked degree, of arsenic. The action is retarded temporarily by the presence of alcohol, but after the latter is absorbed, which occurs very rapidly, there is an increased flow of very active gastric juice.

General Summary.—The main object of the gastric digestion is probably to prepare the food for the pancreatic digestion. The animal food is disintegrated in the stomach, and the myosin and easily digested portions are dissolved to albumose. This sets free the harder fibres and the fat, which latter is melted and floats on the surface. As soon as the food is reduced to a smooth, pulpy consistency, the pylorus relaxes a little and allows the acid mass, chyme as it is called, to pass on into the small intestines for further digestion.

CLINICAL TESTS ON THE GASTRIC JUICE.*

During the last few years it has been found of great advantage, when treating patients suffering from diseases of the stomach, to make more or less thorough analyses of the gastric juice which they are secreting.

For this purpose the stomach is first thoroughly washed out with warm water. Some hours afterward a test meal is administered, usually a light breakfast consisting of a roll and a cup of

* Dr. Francis P. Kinnicutt: "Modern Methods of Examination in Diseases of the Stomach," Medical Record, May 24th, 1890.

hot water, or of tea without milk or sugar, or sometimes a heavier meal of bread and meat. A certain time after this meal, an hour in the case of the breakfast, and as much as four or five hours where more food has been taken, the stomach contents are all withdrawn by a stomach tube and, after filtering, are submitted to examination.

Of the various tests which have at various times been suggested, the following are the most important. They include the determination of—

(*a*) Reaction.
(*b*) Total acidity.
(*c*) Presence of free acids and of acid salts.
(*d*) Presence of free hydrochloric acid.
(*e*) Presence of lactic acid.
(*f*) Presence of syntonin (parapeptone) and peptone.

Reaction.—The reaction is usually determined by the use of litmus paper or of a few drops of an indicator like Orange No. 2. It ought to be acid, and the acidity may be due, as before mentioned, to the presence of either free hydrochloric acid or of lactic or other organic acids, or of acid salts.

Total Acidity.—This is determined, after the methods illustrated in Lesson XV., by neutralizing the juice with a standard alkali solution, in the presence of an indicator. The indicator used is generally phenol-phthaleïn. The standard alkali solution, sodic or potassic hydrate, is usually decinormal, *i.e.*, one-tenth as strong as the solutions described on pp. 141 and 144. In other words, 1 c.c. of it is equivalent to 0.00364 gm. of hydrochloric acid, HCl. We usually make the test on 10 c.c. of the juice, and, as but slight correction is needed for the specific gravity, the percentage of acidity of any sample, calculated to HCl, will be very nearly equal to ten times the above fraction, multiplied by the c.c. of standard alkali solution used. Thus, if 6 c.c. of the alkali solution were enough to neutralize 10 c.c. of a sample of juice, the acidity would correspond almost to $6 \times 10 \times 0.00364$ or about 0.21% HCl.

In practice, 10 c.c. of normal gastric juice usually require from 4 to 6½ c.c. of the standard alkali solution.

Presence of Free Acids or of Acid Salts.—To determine whether

the acidity is due to the presence of free acids or not, it is only necessary to add to the juice an excess of calcium carbonate, $CaCO_3$, and notice if the acidity has been diminished or destroyed. As noticed in Lesson XII., the carbonates are decomposed by free acids, however weak. The $CaCO_3$, however, will not be attacked by the acid salts. This is shown in our experiments by the fact that a dilute solution of HCl is quite neutralized by an excess of $CaCO_3$, while the solution of acid sodium phosphate, NaH_2PO_4, formed by the addition of HCl to the hydro-disodic phosphate, is unaffected.

In practice, this test may be made quantitatively, by determining the total acidity both before and after the addition of the $CaCO_3$, or qualitatively, by comparative color tests on the acidity with litmus paper.

Presence of Free Hydrochloric Acid.—This is a very important determination, for the free HCl in the gastric juice is quite as essential as the pepsin itself, and its secretion appears to be not infrequently interfered with in disease.

It is perfectly possible, although not very convenient for the practising physician, to determine the actual percentage of HCl in the secretion. Usually, however, the qualitative tests are enough for his purposes; and of those, two are particularly interesting, namely:

Günzberg's phloroglucin-vanillin test,* and

Boas' resorcin-sugar test.†

Phloroglucin Test.—This test is in some respects slightly preferable to the other, but unfortunately the reagents employed are both expensive and hard to obtain. The solution of phloroglucin and vanillin (2 gms. phloroglucin and 1 gm. vanillin in 30 gms. of absolute alcohol), when warmed with a little very dilute HCl, becomes red, and deposits cherry-red crystals. The test is so delicate that it reacts with less than 1 part of HCl in 15,000 of water. Only a few drops of gastric juice are needed for this test. They should be put in a small evaporating-dish with a drop of the reagent, and gently warmed over a low flame.

* A. Günzberg. Jahresbericht ü. d. Fortsch. d. Thier. Chemie, '87, p. 242.

† Boas, Jahresbericht, '88, p. 177.

For a comparison of these and other HCl tests, see Jahresbericht, '91, p. 239.

Resorcin and Sugar Test.—This is about as delicate as the phloroglucin test, and, like it, is not affected by free organic acids, and is only slightly affected by acid albumins. The reagent (5 gms. resorcin and 3 gms. sugar dissolved in 100 gms. dilute alcohol), when added to a few drops of very dilute HCl in a small evaporating-dish and warmed, also gives a red color.

Presence of Lactic Acid.—This acid is usually present quite soon after the ingestion of food, but, in normal digestion, disappears when the secretion of HCl has reached its maximum. It can be tested for with a very dilute, almost colorless solution of ferric chloride, Fe_2Cl_6, which turns yellow with even a trace of free lactic acid. It is best to compare this yellow color with the color of some of the same solution without the addition of any gastric juice.

Presence of Syntonin, Albumoses, and Peptones.—These are usually simply tested for together with the biuret test. In case any importance is being attached to the presence of the peptones proper in the fluid, it is possible to precipitate out both albumoses and other proteids by saturation with ammonic sulphate, and then the filtrate can be examined for peptones by the biuret test.

Significance of these Tests.—For any detailed discussion of the clinical significance of the various constituents of the gastric juice the student is referred not only to Dr. Kinnicutt's paper, but to numerous articles in the various medical journals.

We ought, however, to remark that in the diagnosis of at least two diseases these tests are of very considerable value. Thus, in cancer of the stomach, the HCl is almost invariably either totally absent or very greatly diminished. Accordingly, if of no direct positive value, the presence of free HCl in a gastric secretion in at all normal quantities is directly opposed to a diagnosis of gastric cancer. On the other hand, in ulcers of the stomach the HCl and the general acidity is usually considerably increased. Hence, the presence of a highly acid juice, attended with previous vomiting of blood and similar symptoms, may be considered as a pretty strong indication of gastric ulcer.

THE PANCREATIC JUICE.

General Properties.—It is not very difficult to obtain the pancreatic juice by means of fistulæ on lower animals, but the properties of the juice, thus obtained, are so much modified by the after-effects of the operation that it is hard to tell the exact character of the normal secretion. We know it to be a clear, viscid fluid, without much odor or taste, and with a decidedly alkaline reaction equivalent, on an average, to about ½% sodic carbonate.

It contains in solution considerable quantities of solid matter (from 2 to 10%), most of which is organic matter. It contains serum albumin in sufficient quantities to form a solid mass on heating, and enough alkali albumin to give a heavy precipitate on the addition of acids.

The important constituents, besides the alkali, are the various unorganized ferments by means of which it is able to complete the digestion of the different classes of food stuffs. There are supposed to be four of these ferments constantly present, the amylopsin for carbohydrates, trypsin for proteids, steapsin for fats, and a rennet ferment which coagulates milk.

Amylopsin.—This ferment, which can be obtained, in a rather impure form, by precipitation with alcohol from a glycerin extract of the pancreas, resembles very closely, both in properties and action, the diastase and ptyalin already mentioned. It acts, however, much more energetically than the ptyalin, and attacks with ease not only hydrated but even raw starch. It produces maltose and dextro-glucose, the latter being formed in somewhat larger proportions than by the other ferments.

Trypsin.—This ferment, which has been isolated by Kühne and others in a comparatively pure state, changes the albumoses, syntonin, and also the undigested but disintegrated proteids and albuminoids present in the chyme into dialyzable peptones.

The action of trypsin differs from that of pepsin in several important particulars. It works best in an alkaline medium corresponding to 0.5% of sodic carbonate, and, while it still acts in a neutral or even faintly acid solution, it is rapidly destroyed in an acid even as weak as the gastric juice. The proteids subjected to it do not swell up and become transparent before dissolving, as

they do in the gastric juice, but gradually corrode away, changing directly from a solid to a liquid form.

The first stage in this process is the formation of alkali albumin, corresponding to the syntonin in the gastric juice. The alkali albumin is then broken down into albumoses, both hemi- and anti-, just as by the action of pepsin, and these again are further digested into hemi- and anti-peptones. This is done very slowly by pepsin, but trypsin breaks down the albumoses very rapidly, and hence can easily complete the digestion begun in the stomach.

The fundamental difference, however, between trypsin and pepsin is that the former can still further break down the hemi-peptones into the nitrogenous but non-proteid organic bodies leucin and tyrosin, and also, although in small quantities only, into naphthylamin. This action of trypsin probably occurs to but a small extent, if at all, in the body, for the peptones are so diffusible that they are probably absorbed into the circulation as soon as formed.

Trypsin is able to digest both anti-albumin and the albuminoid mucin, both of which are unaffected by pepsin. On the other hand, unlike pepsin, it will not digest the fibres of ordinary connective tissue until they have first been converted into gelatin.

Steapsin, or Fat Digestion Ferment.—The exact nature of the action of pancreatic juice upon the fats is not understood, nor has the ferment, to whose presence it is due, been thoroughly isolated. According to the best authorities, however, the pancreatic juice is able to make an extremely perfect and fine emulsion of fat, and besides this it has the power of converting a small amount of a neutral fat into glycerin and free fatty acid. The fatty acid thus formed is rapidly changed to soap by the alkali present, and hence probably helps to emulsify the rest of the fat. It is generally agreed that the fat is absorbed in the form of minute particles, and is not dissolved, like the proteids and carbohydrates, during the process of digestion.

Rennet Ferment.—This ferment is present in the pancreatic juice, and can be extracted from it in company with the trypsin and amylopsin. It acts in much the same manner as the rennet

ferment found in the stomach. Its special importance, however, is not yet understood.

Leucin and Tyrosin.—These bodies have already been referred to as the final products of the tryptic digestion of the hemi-derivatives of albuminous bodies. They can be produced from both proteids and albuminoids by the action of hot acids and alkalies, and of different kinds of microbes, and are also probably formed by some of the little known processes of metabolism in the body.

They have been found, in normal conditions, in the pancreas, spleen, liver, and some other organs of the body; while in diseases, especially those that affect the liver, they appear in consid-



FIG. 11.—CRYSTALS OF LEUCIN (FUNKE).



FIG. 12.—CRYSTALS OF TYROSIN (FUNKE).

erable quantities all over the body and can be recognized in the urine. They are both readily diffusible.

Leucin. $C_6H_{13}NO_2$.—Leucin, when pure, crystallizes in the form of thin, white, glistening plates not very unlike the crystals of cholesterin, and also in little clusters of fine needles, radiating from a centre. When impure, it forms little yellow or brownish lumps and nodules, which look not unlike little drops of oil, and show hardly any crystalline structure.

It has rather a greasy feeling, and is hard to wet with water, but dissolves readily in hot water and to a slight extent in cold. It is soluble in hot alcohol, and dissolves with ease in acids and alkalies, forming salts with both.

Composition.—Leucin belongs to the fatty group of organic compounds. It is really amido-caproic acid, *i.e.*, the fatty caproic

acid, $HC_6H_{11}O_2$, already mentioned on page 60, with an amido group (NH_2) in the place of one hydrogen. Hence, with bases it acts as an acid, forming (amido-) caproates of the metals and basic radicals, while with acids it forms compound ammonium salts.

Tyrosin. $C_9H_{11}NO_3$.—This substance crystallizes in sheaves or bundles of fine, colorless, radiating crystals which sometimes are large enough to show a prismatic form. It dissolves readily in hot water and hot alcohol, but with difficulty in cold water. It also dissolves in both acids and alkalies, and forms salts with them and also with many metals. Some of the latter have been separated and are quite characteristic.

Tyrosin differs in composition from leucin in that, besides a fatty acid and an amido group, it also contains an oxy-phenyl group (C_6H_4OH), and therefore must be classed with the aromatic compounds. The presence of both leucin and tryosin in the decomposition products of proteids tends to show that in the latter some of the carbon atoms are arranged as in the benzol ring, and some in chains as in the fatty compounds.

Tyrosin responds very readily to the Millon's reaction.

LABORATORY EXPERIMENTS.

GASTRIC, PANCREATIC, AND SALIVARY DIGESTION.

I. Tests on Artificial Peptones.

Prepare the dialyzer by tying a piece of wet parchment paper over one rim of the glass ring, so as to make a water-tight cup of it. Suspend this, from the filter stand, in a beaker containing about three inches of water, so that the paper bottom of the dialyzer sets into the water about half an inch. Place the peptone solution inside the dialyzer and let it stand quietly until the end of the lesson. Then test the liquid inside the dialyzer for albumoses as follows:

(*a*) Boil a little of the solution in a test-tube = no ppt.

(*b*) Add about an inch of dry NaCl to some of the solution in a test-tube = ppt.

(*c*) Acidify a little in a test-tube with a drop or two of acetic acid, $HC_2H_3O_2$, add a few drops of potassic ferrocyanide, K_4FeCy_6, and boil = ppt.

(*d*) Add about an inch of crystals of ammonic sulphate, $(NH_4)_2SO_4$, to some in a test-tube, and heat = ppt.

(*e*) Add to some in a test-tube a few drops of a solution of tannic acid = ppt.

(*f*) Test some with the biuret test = violet.

Test the liquid outside the dialyzer for peptones as follows:

(*a*) With $(NH_4)_2SO_4$ crystals = no ppt.

(*b*) With tannic acid = ppt.

(*c*) With picric acid = faint cloudiness.

(*d*) With biuret test = reddish color.

II. **Gastric Digestion.**—Test the digestive power of the artificial gastric juice as follows:

Reaction.—Put a little fibrin in a large test-tube, fill it nearly full of gastric juice, and place the tube, carefully labelled, in the agate cup half full of water. Let the test-tube rest on a piece of wire gauze set into the cup about an inch from the bottom. Place the cup on a sand bath and warm very gently, testing it constantly with a thermometer until it is between 38° and 40° C. Let the mixture of fibrin and gastric juice stand at this temperature for an hour, stirring it every now and then. Notice that the fibrin first swells up and becomes more or less transparent (see Syntonin, Lesson IX.), and finally dissolves, under the action of the pepsin and hydrochloric acid, to albumoses and, in process of time, to peptone. When it is all dissolved, make the following tests on the liquid, in three different test-tubes:

For Albumoses.—Add $(NH_4)_2SO_4$ in crystals = ppt.

For Albumoses and Peptones.—Add some solution of tannic acid = ppt.

For Peptones.—Try carefully the biuret test. If peptones have been formed in any abundance, the color will be reddish or rose-color.

III. **Clinical Tests on Gastric Juice.**—While waiting for the completion of the gastric and pancreatic digestion experiments, make the following tests upon what is left of the artificial gastric juice:

1st. *Reaction.*—Determine the reaction with a piece of litmus paper.

2d. *Total Acidity.*—Place in a small beaker exactly 10 c.c. of the liquid, add a drop or two of phenol-phthalein, and run in carefully from the burette the standard alkali solution, stirring the liquid constantly, until the mixture just turns pink. Read off the number of c.c. of alkali solution used, usually from 4 to 6½. To calculate the percentage of acidity, expressed in terms of free HCl, multiply ten times the number of c.c. used by the factor 0.00364.

3d. *Presence of Free Acids and of Acid Salts.*—Add to 10 c.c. of the juice about half a teaspoonful of powdered $CaCO_3$. Mix thoroughly, and, after any effervescence has ceased, filter the mixture into a small beaker, washing the precipitate two or three times on the filter with water from the wash bottle, and letting the washings drain into the same beaker. Then add to the filtrate a drop or two of phenol-phthalein, and determine the acidity as above, by titrating with decinormal standard alkali solution. Any acidity thus found will be due to acid salts, while the difference between the former determination and this will be due to free acid.

To illustrate the action of the $CaCO_3$, make up two solutions: one of free HCl, by adding two or three drops of HCl dil. to some 25 or 30 c.c. of water; and the other of acid sodium phosphate, NaH_2PO_4, by adding to a little solution of Na_2HPO_4 enough HCl dil. to just make it distinctly acid to test paper. To each of these solutions add half a teaspoonful or so of $CaCO_3$, and after the effervescence has all ceased test each with litmus paper. Notice that the HCl solution is now neutral, while the NaH_2PO_4 solution still remains acid.

4th. *Tests for Free HCl.*—(*a*) *Phloroglucin Test.*—Put in a small evaporating-dish four or five drops of the juice, and to them add one drop of the phloroglucin-vanillin solution. Warm gently over a low flame, and notice the streaks of red which appear along the sides and on the surface of the mixture.

(*b*) *Resorcin Test.*—Put in a small evaporating-dish four or five drops of the juice, and to them add one or two drops of the

resorcin-sugar solution. Warm gently over a low flame and notice the reddish color which results.

5th. *Test for Lactic Acid.*—Make an exceedingly weak solution of Fe_2Cl_6 (one drop of the latter to 25 c.c. or so of water) and place it in two test-tubes. To one add two or three drops of the juice and notice if its color turns more yellow than the color of the liquid in the other tube. Repeat this test until you have found the right strength of Fe_2Cl_6 to employ.

IV. **Pancreatic Digestion.**—Test the pancreatic juice as follows:

1st. *Reaction.*—Notice that it is alkaline to test paper.

2d. Na_2CO_3.—Notice that a little of it, in a test-tube, effervesces when a drop of HCl is added.

3d. *Action of Trypsin on Fibrin.*—Put a little fibrin in a large test-tube, fill it nearly full of pancreatic juice, and place the tube, carefully labelled, alongside the tube containing the fibrin in gastric juice, in the agate cup. Let it stand there, at a temperature between 38° and 40° C., for an hour, stirring occasionally. Notice that the fibrin does not swell up or become transparent, like the other, but gradually wastes away.

At the end of an hour or so test the liquid as above, under Gastric Digestion, for albumoses, for albumoses and peptones, and for peptones.

4th. *Action of Trypsin and of the Rennet Ferment on Milk.*—Fill a large test-tube nearly full of milk and add about half an inch of the juice. Label the test-tube, place it in the cup, and keep it at a temperature between 38° and 40° C. for an hour. Notice that the milk coagulates in a few minutes, but that the coagulum soon redissolves. At the end of the hour test the milk as follows:

(*a*) Taste it; notice the peculiar bitter taste due to peptones.

(*b*) Add a drop or two of HNO_3 dil.; notice that the casein can be no longer coagulated.

(*c*) Try the biuret test carefully; notice that it gives a reddish rather than a violet color, owing to the presence of peptones.

Make the following tests on the Glycerin Pancreas Extract:

Action of Amylopsin.—Make some starch paste, as in Les-

sons I. and III., and put some in two small evaporating-dishes. Let them cool till the paste is at about 40° C., and then to the 1st add a few drops of the extract, and to the 2d add a few drops of the extract mixed with two or three drops of HCl dil. Warm both for a few minutes, but *not over* 40° *C.* Then test each for maltose with dilute Fehling's solution, and by the picric-acid test. The first paste will show maltose, but the second will not.

V. **Salivary Digestion.**—1st. *Microscopical Appearance.*—Examine a drop of saliva under the microscope. Notice the epithelial cells and débris, bacteria, scraps of food, etc.

2d. *Potassic Sulpho-Cyanide.*—Put a little saliva in a small test-tube and add a drop of much diluted Fe_2Cl_6. In most instances there will be formed a slight reddish color. Compare this with the color formed in the same amount of water, in another test-tube, by the same amount of Fe_2Cl_6.

3d. *Reaction.*—Notice that, excepting in rare cases, it is perceptibly alkaline to test papers.

4th. *Action of Ptyalin.*—Put some starch paste into two small evaporating-dishes. To the first add a little saliva; to the second add a little saliva mixed with one drop of HCl dil. Warm both for a few minutes at not over 40° C., and then test each for maltose with dilute Fehling's solution. The first will show maltose, but the second will not.

VI. **Leucin and Tyrosin.**—Test a little of the leucin and tyrosin solution as follows:

Put one or two drops on a glass slide and let them evaporate. Notice the yellow or brownish, round crystals of leucin, and the radiating, needle-shaped crystals of tyrosin.

PART VIII.

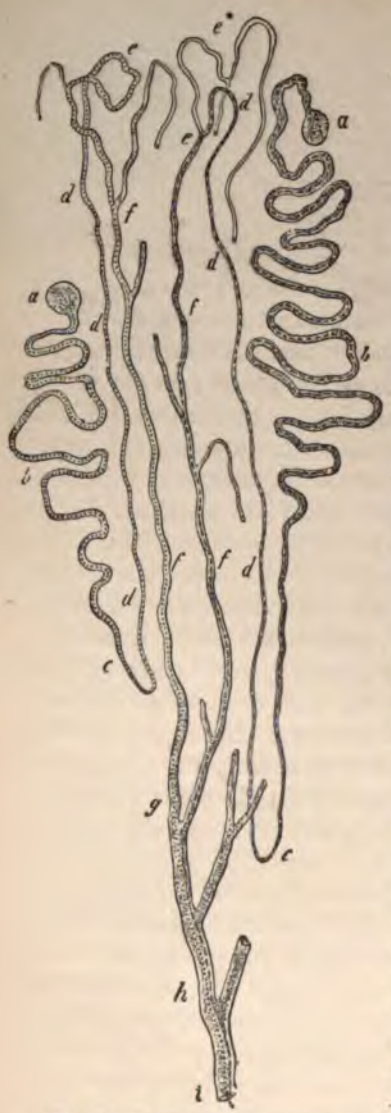
THE URINE

THE URINE.

INTRODUCTION.

THE various foodstuffs, which enter the system in the form of carbohydrates, proteids, albuminoids, and fats, are all utilized, sooner or later, for the production of manifest energy, by a process of oxidation. Most of the carbon atoms pass out of the body through the lungs in the form of carbon-dioxide. The greater part of the hydrogen is oxidized to water, and is excreted through both lungs and skin, as well as through the kidneys; while the nitrogen, oxidized to urea and its allies, with the mineral salts which enter the body in the food, and the sulphates and phosphates resulting from the oxidation of the proteids and albuminoids, are eliminated from the body almost entirely in the urine. These nitrogenous waste products are distinctly injurious to the system, if retained in any quantity, and their excretion is the duty of the kidneys. Besides this, however, the kidneys have the further duty of excreting most of the abnormal and superfluous substances which at any time enter the system, and also, by being able to separate water from the blood when occasion calls for it, they regulate the density and hence the quantity of the blood in the body.

Structure of the Kidney Tubules.—The urine is derived from the blood in those portions of the kidneys known as the uriniferous tubules. These are exceedingly minute, slender tubes, leading, by means of larger and larger channels, into the pelvis of the kidney, which communicates with the bladder through the ureter. These tubules have a very long, winding course before they finally reach the pelvis, and are lined their whole length with a continuous layer of highly developed epithelial cells. The latter vary greatly in size, in shape, and, it is supposed, in functions, according to the part of the tubule in which they lie.



The tubules originate in what are known as Malpighian bodies, cup-shaped enlargements into each of which is inserted a little tuft of blood-vessels known as the glomerulus. The blood-vessels of the glomerulus are separated from the interior of the tubule only by a delicate layer of flat epithelial cells, thus affording an opportunity for water and for diffusible matter in the blood to pass through into the tubule. After the blood has left the glomerulus, it does not directly leave the kidney, but passes in a series of fine blood-vessels round and round the tubules on their way to the pelvis, and thus gives another opportunity for the execretion of urinary ingredients.

Formation of the Urine.—The actual process of secretion of the urine in the tubules is not at present fully understood, for the reason that it undoubtedly does not depend upon diffusion alone. Indeed, it is generally agreed that the cells which cover many portions of the tubule

FIG. 13.—DIAGRAM REPRESENTING THE COURSE OF THE URINIFEROUS TUBES, BASED ON THE ARRANGEMENT AS SEEN IN THE KIDNEY OF THE PIG. *a*, Bowman's capsule; *b*, convoluted uriniferous tube; and *c*, recurrent arm of loop; *d*, descending arm; *e*, convoluted passages; *f*, collecting tubes joining to form one large, open uriniferous canal, *g*, which communicates with another canal, *h*; *i*, main trunk opening on the papilla (Frey).



are of the nature of true secreting cells, as in the various other glands of the body, salivary, gastric, mucous, and others; and also, that among their special functions is that of ridding the system of urea and other nitrogenous waste products. The urea is probably formed elsewhere in the body, and is simply removed from the blood by the tubule cells, but some of the other nitrogenous bodies may be very possibly elaborated by them as well as excreted.

The epithelial covering of the glomeruli, which is extremely thin and delicate, probably acts more as a dialyzing medium, allowing water and some of the diffusible compounds of the blood, especially inorganic constituents and, in abnormal conditions, glucose or peptones, to pass through. But even here the cells do not act simply like a sheet of parchment paper. They seem to discriminate between the different crystalloids, as for instance in not letting the urea through, and also between colloid bodies; for while serum albumin, in perfectly normal conditions, never passes from the blood into the urine, egg albumen, if injected into the system, is rapidly excreted by the kidneys, probably through the glomeruli.

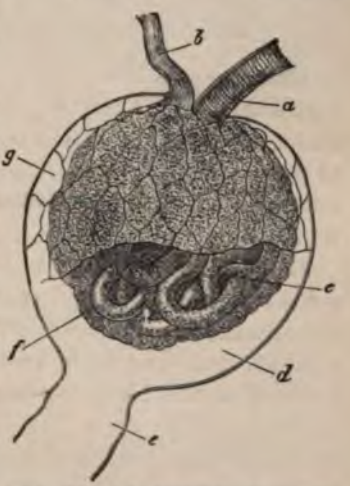


FIG. 14.—A GLOMERULUS FROM THE RABBIT. *a*, vas afferens; *b*, vas efferens; *c*, glomerulus; *d*, undermost portion of capsule without epithelium; *e*, neck; *f*, epithelium of the glomerulus; and *g*, that of the internal surface of the capsule after treatment with nitrate of silver (Frey).

It is interesting to notice that, while the formation of urine cannot be considered as simply one of diffusion, still the only constituents of the blood which pass under normal conditions into the urine are, like urea and the different salts, organic and inorganic, distinctly crystalloid bodies. When, however, the nutrition of the cells is impaired, by disturbances of the circulation which nourishes them or by actual disease, one of the symptoms noticed is the presence in the urine of albumin, paraglobulin, and other non-diffusible substances.

LESSON XXII.

GENERAL PROPERTIES OF THE URINE.

Quantity of Urine.—The amount of urine passed in twenty-four hours by a healthy individual varies within quite large limits, depending upon the relative amount of fluid imbibed and of water given off through the lungs or skin. For an adult the average amount is usually estimated at from 1,200 to 1,500 c.c. (40 to 50 fluid oz.).

But besides the physiological variations, many diseases have a decided influence upon the quantity of urine excreted. Thus, the quantity of urine is often diminished in disorders of the circulation, and in acute, or sometimes in chronic, nephritis or inflammation of the kidneys. In fevers, also, less urine is usually passed.

Its quantity is increased in diabetes, both insipidus and mellitus, and in some kinds of heart and kidney disorders.

The quantity of urine can usually be increased by increasing the amount of fluid imbibed. It can also be increased, in many cases at least, by the use of certain drugs, known as diuretics, such as citrate and other salts of potash, nitrous ether (sweet spirits of nitre) and others.

The determination of the exact amount passed in twenty-four hours is often of great importance in certain diseases not only for its own sake, but also because, unless it is known, it is impossible to determine the amount of urea excreted from the body.

Specific Gravity.—The density of urine, which averages for healthy individuals somewhere between 1.015 and 1.025, varies, in general, inversely with the quantity. Hence, in conditions of perfect health it is quite possible to find it as high as 1.045 and as low as 1.002.

In disease, this rule does not always hold good. The urine, for instance, in diabetes mellitus is not only excreted in great

abundance, but is also, in most cases, very heavy from the presence of glucose. In certain kinds of nephritis, on the other hand, the urine, which is diminished in quantity, may also be quite light on account of its small percentage of urea.

The specific gravity of a sample of urine ought always to be determined, not only as being of value in detecting certain disorders, but also as giving some general information about the solid constituents of the urine. The instruments known as urinometers, which are used for this purpose, are simply small hydrometers, graduated to read specific gravity directly, with a range of from 1.000 to 1.050 or 1.060. It is always well to test the accuracy of a new instrument by placing it in distilled water and noticing if it stands at the 1.000 mark.

Color.—This also, as a rule, is dependent upon the degree of concentration of the urine, and varies, under normal circumstances, from a very faint yellow to a rather deep brown color. The pigments of the urine have not yet been thoroughly isolated, but it is believed that they are derived from the bilirubin and other coloring matters of the bile.

The color of the urine is often influenced by disease, independently of the quantity excreted. Thus, it is lighter, not only in diabetes, as we should expect from the increased flow, but also in certain kinds of chronic nephritis and in anæmia.

It is perceptibly darker in congestion of the kidneys, and also in fevers, where it is claimed that additional coloring matters make their appearance. It is also often colored by the presence of blood which gives it a reddish tinge, deepening often, on standing, to a dark brown, or of bile pigments which give it a yellow or even greenish tinge.

The color of urine is also affected by the use of certain drugs. Thus, large doses of salicylic acid will make it green, while carbolic acid makes it dark green to black, and rhubarb, brown or red.

Consistency.—Normal urine is always aqueous, but not infrequently, in disorders of the genito-urinary tract, it is thick and more or less viscid or ropy. This is quite common in severe inflammation of the bladder, and is due either to the excessive presence of mucus, or to the action of alkaline, decomposing urine upon pus.

Reaction.—Fresh urine is usually more or less acid from the presence, not of free acids, but of acid salts, principally acid sodium phosphate, NaH_2PO_4, and, perhaps, acid salts of uric acid. It is never perfectly neutral, but while it may occasionally be amphoteric, it is not infrequently perceptibly alkaline owing to the presence of alkaline phosphates and carbonates. This often happens during digestion.

After urine has stood for some time at ordinary temperatures the urea present is decomposed by certain bacteria, of which the Micrococcus ureæ of Cohn is the best known, into ammonium carbonate, according to the formula

$$\underset{\text{Urea.}}{(NH_2)_2CO} + 2H_2O = \underset{\text{Ammonium Carbonate.}}{(NH_4)_2\,CO_3}$$

This makes the urine decidedly ammoniacal in both smell and reaction. It also always produces more or less turbidity, not only from the presence of the bacteria themselves, but from the precipitation of earthy phosphates, triple phosphate, and similar compounds insoluble in alkaline solutions.

The reaction of the urine can be readily influenced both by drugs and by diet. Thus the urine may be made alkaline, not only by actual doses of carbonate of soda and other alkalies, but also by simply diminishing the amount of meat taken, and by feeding upon vegetable food, and especially upon fruit. The latter contain quite a large amount of the organic salts of soda and potash, citrates, malates, tartrates, and the like, which seem to be oxidized in the system to carbonates.

Most diseases have but little effect upon the reaction of the urine. In fevers, however, it is generally more acid than is customary; while the passage of alkaline, ammoniacal urine indicates decomposition of the urine and the presence of microbes in the bladder—a condition usually accompanied with considerable inflammation of that organ.

Transparency.—Urine is never perfectly transparent, and it may, under perfectly normal conditions, be decidedly turbid.

Mucus.—There is always present, in every sample of urine, more or less mucus from the lining of the bladder and urinary passages. This is usually exceedingly slight, but it can generally

be noticed, especially after standing a few minutes, as a faint cloudiness in some part of the liquid. It is unaffected by heat, alkalies, or even acids, although the latter occasionally increase the turbidity slightly by precipitating the mucin.

Phosphates.—If the urine is alkaline, either naturally or from undergoing the ammoniacal fermentation, there is always a deposit of the earthy phosphates, and, in the latter case, of ammonio-magnesio or triple phosphate. These compounds usually settle to the bottom in a short time, and are readily distinguished, 1st, by appearing only in alkaline urine; and 2d, by dissolving when the urine is acidified with acetic acid.

Urates.—If, however, the urine is acid and happens to be concentrated, a deposit is very apt to occur, on lowering the temperature, composed of urates of sodium, potassium, calcium, and magnesium. These are usually colored yellow or even red, and form the common brick-dust deposits so frequently met with in chilled urine. They are readily recognized, 1st, by only occurring in acid urine; 2d by dissolving on warming; and 3d, by dissolving when the urine is made alkaline.

Microbes.—Although the normal urine is almost always sterile as it leaves the bladder, it is invariably mixed with germs if allowed to stand without special precautions, and hence, unless specially treated, is bound to undergo decomposition. Both mould and yeast plants frequently grow in it, and can be recognized readily under the microscope; while the bacteria multiply in it with great rapidity, and are so abundant in stale urines that they often make it quite cloudy. These germs cannot be removed by filtering through paper, and can be recognized, even without the aid of the microscope, by not being dissolved by heat, acids, or alkalies, by the liquid not clarifying on standing, and by the odor and reaction of the urine.

The presence of bacteria in any abundance, especially if accompanied by triple phosphates, in the perfectly fresh urine, is often an indication of disease in the bladder.

Pus.—Besides this, in inflammations of the genito-urinary tract, the urine is often made more or less turbid by the presence of pus. These pus cells frequently can only be recognized by careful examination under the microscope, but when in great

abundance they may appear as a yellowish-white cloud or sediment. They are also not infrequently found in strings and threads, bound together by mucus. On the addition of potassic hydrate, or in the presence of ammonia, the pus cells can be converted into alkali albumin, forming a thick, transparent, ropy deposit.

Epithelial Cells and Débris.—These, as well as the pus cells, can only be properly identified under the microscope, and therefore will be discussed later.

Fat Globules.—Fat occasionally occurs in the urine as minute globules contained in epithelial cells, or, very rarely, in casts, in cases of fatty degeneration of the kidneys, and sometimes in other disorders.

There is, however, besides this a condition, known as chyluria, in which the urine contains quite large quantities of fat globules mixed with albumin, cholesterin, and other substances. These may give the urine quite a milky appearance. The fat globules can be recognized, not only by the microscope, but also by their rising to the surface on standing. This condition is due to the passage of lymph into the urine through channels made, in many cases at least, by the action of parasites.

Odor.—The odor of normal urine is perfectly characteristic, although it varies considerably in intensity according to the concentration. It is usually designated as aromatic, or simply as urinous. After the urine has undergone decomposition, it smells strongly of ammonia, and at the same time usually has a putrid smell from the action of the microbes upon the mucus and other organic matter present.

This latter odor is occasionally observed in diseases of the bladder and kidneys, while in cases of diabetes mellitus the urine now and then has a sweet or fruity smell, and, after standing a little, develops a distinct odor from the setting in of alcoholic fermentation.

The odor can easily be influenced by certain drugs, such as cubebs, copaiba, oil of sandalwood, turpentine, and others; and also, to a marked degree, by certain vegetable foods, as, for instance, by asparagus.

GENERAL CHEMICAL PROPERTIES.

The urine is the great channel for excreting from the system either foreign or superfluous solid material. Hence, its composition is extremely complex, and varies considerably, not only with the amount and quality of the ingesta, but also with the course of metabolism in the body itself.

It usually contains, when passed in quantities of 1,200 to 1,500 c.c., some 4 to 6% of solid matter, of which rather more than half is organic. This organic matter is chiefly composed of the crystallized nitrogenous compounds, urea, uric and hippuric acids, and their salts, which represent the nitrogenous waste of the system. Besides these, there are small quantities of pigments, and also, it is believed, of unorganized ferments and of non-nitrogenous bodies, which have not yet been satisfactorily isolated.

Special attention will be given in the next lesson to urea and uric acid.

The urates, which are always present to some extent, are, as already mentioned, not very soluble in cold acid urine, and can be precipitated, if in excess, by simply cooling the acid urine down to a low point. This test, although very rough and simple, is often made in practice, because an excess of urates is considered to indicate in many cases a predisposition to rheumatism and gout.

The inorganic constituents consist, as in the case of blood, milk, and bone, of the chlorides, sulphates, and phosphates of the alkaline and earthy metals, sodium chloride being in considerably greater quantities than the rest.

Of the inorganic constituents, the sulphates and phosphates are of but slight importance. The chlorides, however, are usually present in normal urine to a very large extent, forming quite a heavy, curdy precipitate with $AgNO_3$. In severe illnesses they usually diminish, and their return in normal amounts, which occurs in the early stages of convalescence, is often watched for and regarded as a favorable symptom.

LABORATORY EXPERIMENTS.

THE GENERAL PHYSICAL AND CHEMICAL PROPERTIES OF URINE.

The student will be provided, in the following lessons, with a number of specimens of urine, both normal and pathological. The latter will, if possible, include specimens from diseases, such as nephritis, cystitis, gonorrhœa, diabetes and the like, which have more or less marked influence on the urine.

The student will be expected to examine carefully as many of these samples as possible, making note, in each case, of the disease, and then carefully recording for each the results of his examination, conducted as follows.

I. **Specific Gravity.**—Test this carefully with the urinometer, reading it always at the top of the meniscus, *i.e.*, the little ring of liquid that forms round the stem of the instrument.

II. **Color.**—If the color is normal, note whether it appears light, medium, or dark. If the color varies, describe it as accurately as you can.

III. **Consistency.**—Note if it is anything but a thin, aqueous liquid.

IV. **Reaction.**—Note if it is acid or alkaline to test paper.

V. **Transparency.**— Examine carefully and notice if there is more than a faint cloudiness, due to mucus. If there is, decide whether it is due to

(a) *Mucus.*—This forms in clouds, and is unchanged by acids, alkalies, or heat.

(b) *Earthy and Triple Phosphates.*—These occur only in alkaline urine, and the latter only after decomposition. They dissolve on acidifying.

(c) *Urates.*—These occur only in acid urines; they dissolve on warming and on the addition of alkalies.

(d) *Bacteria in Large Quantities.*—These occur only in de-

composing urine, which can be recognized by the smell. They make the whole liquid cloudy, and also cause, generally, a precipitation of phosphates. They cannot be filtered out, and are unaffected by heat, acids, and alkalies.

Also in pathological urines—

(e) *Pus.*—This, when in great abundance, may form a creamy white deposit or cloud, or else may occur as white strings and threads.

(f) *Epithelial Cells and Débris.*—These, when in abundance, often look much like pus, although often having a red or brownish color. They can only be properly recognized, like most of the other causes of turbidity, by the microscope.

(g) *Fat Globules.*—Very rarely present. They rise to the surface on standing and make the urine milky.

VI. **Odor.**—Notice whether it is of the ordinary aromatic type, and whether it is weaker or stronger than usual. Also notice whether it smells putrid, or ammoniacal, or sweet.

VII. **Urates in Excess.**—If the specific gravity of the urine is at all high, 1.025 and over, test whether the urates are in excess by putting some of it in a test tube, cooling it rapidly under a stream of cold water, and noticing if a precipitate occurs.

VIII. **Chlorides.**—Test for these by acidifying half a test tube full of urine with HNO_3, filtering off any precipitate that may occur, and then adding to the filtrate half an inch or so of $AgNO_3$.

In normal urines the resulting precipitate of AgCl is quite heavy, either curdy or lumpy. If the mixture only becomes milky, the chlorides are below the average.

IX. **Other Inorganic Constituents.**—Test for these as follows:

(a) *Sulphates.*—Add to the urine $HCl + BaCl_2$ = white ppt.

(b) *Earthy Phosphates.*—Make the urine alkaline with NH_4OH = white or gray ppt.

(c) *Alkaline Phosphates.*—Filter off the ppt. from (b), and to the filtrate add some NH_4Cl and then some magnesic sulphate $MgSO_4$ = white or gray ppt.

N. B.—At the end of this lesson set aside in a conical glass some urine strongly acidified with HCl.

LESSON XXIII.

UREA AND URIC ACID.

These are the most important substances that are found in the urine. They are the end products of the oxidation of the proteids and albuminoids of the body, and if for any reason their excretion is hindered and they are allowed to accumulate in the system, serious results are liable to occur.

Urea is most prominent in the urine of the mammalia, while uric acid and its salts form the chief constituents of the excretions of birds and reptiles. They both occur together, in the human urine, in proportions which differ considerably according to conditions not yet understood. On an average, however, it is probable that in health about 87 per cent, and in fevers about 83 per cent, of the nitrogen in the urine is excreted in the form of urea.

UREA. $(NH_2)_2CO$.

Occurrence.—Urea is the principal constituent of the urine of man and of carnivorous animals. It occurs to a considerable extent in the urine of the herbivora, and, though far less abundantly, in the excretions of birds, reptiles, and fishes. It is found in small quantities in the blood, milk, and some other fluids of the body.

Quantity.—Urea is excreted from the body at an average rate, in the case of a well-fed, healthy man, of from 20 to 35 grammes (¾ to 1¼ oz.) in twenty-four hours. This quantity varies very considerably, not so much with the amount of exercise, as with the amount of nitrogenous food digested. In perfect health it has been known to range, according to the food supplied, from under 10 gms. to over 100 gms. per twenty-four hours.

It is noticed, under pathological conditions, that in fever the excretion of urea may increase very considerably, even when no nitrogenous food has been administered. On the other hand, in

severe diseases of the kidneys, the amount of urea excreted in the urine may fall far below what would be expected from the diet and general condition of the patient. In this connection it is important to remember that the skin may be called upon, by thorough sweating, to assist the kidneys in getting rid of some of the urea. If the excretion is seriously interfered with for any length of time the condition known as uræmia may set in, and may be attended with very serious consequences.

Formation of Urea in the Body.—It is at present believed that the urea is formed not in the kidneys, but elsewhere in the body, very possibly in the liver, and then is carried in the blood to the tubule of the kidney, where it is removed from the system. Various bodies, such as leucin and also kreatin, have been suggested as the intermediate products between the muscle tissue and urea.

Preparation.—Urea can be prepared synthetically by heating together potassic cyanate and ammonic sulphate. The ammonic cyanate, thus formed, is converted, during the operation, into urea by a rearrangement of the atoms in the molecule. Thus

$$NH_4CNO = (NH_2)_2CO.$$

Urea can also be extracted from urine which has been evaporated down to a thick syrup, by adding nitric acid, filtering off the crystals of urea nitrate thus formed, and decomposing them with $BaCO_3$ into urea and baric nitrate, $Ba(NO_3)_2$. The urea can be extracted from the mixture by hot alcohol.

Properties. (a) *Physical.*—Urea, when pure, crystallizes in colorless rectangular prisms, belonging to the rhombic system.

Its crystals are anhydrous, and melt at 130 to 132° C. It has a bitter cooling taste, resembling that of saltpetre. Water and also alcohol readily dissolve it, forming neutral solutions, but it is insoluble in pure ether and chloroform.

(b) *Chemical.*—The chemical structure of urea, or carbamide as it is called in chemistry, will be readily understood on remembering that it is an amide of carbonic acid, *i.e.*, that it is carbonic acid with two NH_2 groups in place of two OH groups.

Thus $\begin{matrix} H-O \\ H-O \end{matrix}\!\!\rangle C=O$ $\qquad$ $\begin{matrix} H_2N \\ H_2N \end{matrix}\!\!\rangle C=O$

Carbonic Acid. $\qquad$ Urea.

Urea may also be considered as a compound where two ammo-

nias have one hydrogen in each replaced by one bond of CO. For this reason it forms with acids, both organic and inorganic, a series of crystalline salts, two of which, the nitrate and oxalate, are less soluble, and hence better known than the rest. The nitrate of urea, which can be easily formed by acidifying concentrated urine with HNO_3, crystallizes in white, four or six sided, rhombic plates. These usually overlap each other more or less, and while quite soluble in water are comparatively insoluble in dilute nitric acid. The oxalate of urea forms colorless, rhombic crystals with sharply defined faces. It dissolves but slightly in both alcohol and water.

Urea also forms a series of compounds with many metallic salts, such as NaCl, NH_4Cl, the chlorides and nitrates of Hg (ic), Au, Zn, Cu, etc. These substances are usually crystalline, but vary greatly in solubility, the compound formed with palladium chloride being particularly insoluble.

The compound with NaCl is an interesting example of these bodies. It crystallizes in colorless, flat plates, which dissolve without difficulty in water.

The compound with mercuric nitrate is of importance as the basis of Liebig's well-known method for determining the percentage of urea, by the use of a standard solution of $Hg(NO_3)_2$. The end of the reaction is known by observing when the mixture of urine and mercuric salt form a yellow precipitate, on treating a drop of it with alkali. The compound that is usually formed in this reaction has a composition of $2N_2H_4 . Hg(NO_3)_2 . 3HgO$. It forms a heavy, white, crystalline precipitate, which is soluble in dilute nitric acid and in solutions of NaCl.

Quantitative Determination of Urea. Importance.—In many, if not most, cases of kidney disease the insufficient excretion of urea is the main danger to be guarded against. Hence it might be supposed that the determination of the urea would frequently be of very great importance. In practice, however, the analysis is not made as often as might be expected, for two reasons. In the first place, the mere determination of the percentage present in a given sample is worthless, unless the quantity of urine passed in twenty-four hours is known also. And, secondly, even when this is done, and the actual quantity of urea passed in a given

time is known, the figures are of little value unless at the same time we can estimate, with some accuracy, how much urea *ought* to pass in that same period.

In other words, it is not the actual amount but the relative amount of urea that is of interest, and a patient might be more liable to an attack of uræmia while passing 30 gms. (an oz.) a day, when in a state of high fever, or when consuming large quantities of meat, than another passing only half that amount, when living quietly on a strictly carbohydrate diet.

Methods.—(*a*) *Specific Gravity.*—It is often possible to make a rough guess at the percentage of urea from the specific gravity of a sample, if we know, also, the quantity of urine passed, and the comparative abundance of the chlorides. In an average sample of urine the quantity may be set at 1,400 c.c., the specific gravity at 1,020, and the amount of urea at 2½%. The main constituent of urine, besides urea, is sodium chloride, and if that is fairly constant a variation in the specific gravity would indicate a corresponding variation in the amount of urea, and a density of 1.015 might indicate from 1 to 1½%, and of 1.030, from 3 to 3½% of urea. In the presence, however, of other disturbing factors, such as albumin or glucose, even this rough guessing becomes impossible.

(*b*) *Liebig's Method.*—This has already been mentioned. The results from it are fairly accurate and not hard to get, but it has been superseded of late years by the more convenient and equally accurate hypobromite method.

(*c*) *Hypobromite Method.*—This depends upon the fact that when urea is oxidized the hydrogen is converted into H_2O and the carbon into CO_2, but the nitrogen remains unchanged. Hence, if the oxidation is done in a strongly alkaline medium, which will absorb the CO_2, the only gas which will be given off will be pure nitrogen, each c.c. of which corresponds to 0.00282 gms. of urea.

The solution used to oxidize the urea is a strongly alkaline solution of bromine. It is usually made by dissolving 100 gms. of NaOH in 250 c.c. of water, and adding 25 c.c. of bromine to this solution. The solution does not keep for more than a few days, so it has been suggested to make up the solution of NaOH by

itself, and to fill the apparatus with this each time, adding the proper quantity of bromine (for the Doremus apparatus 1 c.c. is enough) with a pipette just before using.

Several varieties of apparatus have been introduced for making this test. In our experiments we have used the simple apparatus devised by Dr. C. A. Doremus,* and also the more elaborate one of Dr. John Marshall. †

It will be noticed that the little Doremus apparatus is much simpler, and is more quickly and easily managed, than the other; while, if it is used carefully, the results obtained by its use are almost identical with those obtained by Dr. Marshall's method. Another great advantage of the former is that no calculation is needed. It requires, however, very careful manipulation.

Functions in Nature.—The urea is purely a waste product, which must be gotten rid of from the body as quickly as possible. It is interesting to notice that while the carbon and hydrogen are thoroughly oxidized in the body by conversion into CO_2 and H_2O, the nitrogen leaves the body in a very slightly oxidized condition, and that hence the body seems to waste, in the form of urea, a considerable amount of potential energy.

URIC ACID. $C_5H_4N_4O_3$.

Occurrence.—Uric acid is always present, in small amounts, in the human urine, and in the urine, not only of carnivorous, but generally also of herbivorous, animals. It is produced in quite large quantities by the lower animals, and the excretions of birds and of most reptiles are almost entirely composed of uric acid and ammonium urate. Hence it occurs in large quantities in the deposits of guano found in dry countries.

Preparation.—Uric acid can be prepared pure by acidifying an aqueous solution of snake's or bird's excrement. It can also be prepared, although in an impure state, by making urine strongly acid, and letting the uric acid crystallize out. It sometimes crystallizes from urine naturally, after standing a little while.

Properties.—When pure it is a fine, light, white powder, which under the microscope is seen to consist of colorless, rhombic crys-

* Medical Record, March 14th, 1885. † Zeits. f. Physiolog. Chemie., xi., p. 179.

tals, see Plate VI. It dissolves very slightly in water, the impure varieties being more soluble than the pure crystals.

It is insoluble in alcohol and ether, but dissolves in glycerin. Its solutions in the cold do not have an acid reaction.

It is readily soluble in alkaline solutions, forming salts, urates, which can be precipitated by cold and by acids, but which can be redissolved on warming or by alkalies. It is decomposed by the hypobromite solution just the same as urea, and hence is almost always estimated with the latter, when the urine is analyzed.

Tests.—The prettiest test for the presence of uric acid is the formation of a red or purple coloring matter, known as murexide, when the compound is first oxidized with nitric acid, and then evaporated to dryness with the addition of a drop of dilute ammonia. This test is often of importance in determining the composition of a piece of calculus.

Functions in Nature.—Uric acid, on oxidation, yields urea and an organic acid, and hence is often spoken of as a product of insufficient oxidation of the nitrogenous matter of the body. It is probable, however, that it is a true end-product of metabolism in the body, only that for some reason it has been formed instead of urea. Indeed, while in the higher animals uric acid, taken into the system, is excreted as urea, urea that is fed to birds is excreted as uric acid.

The actual amount of uric acid and of its salts excreted from the body depends, so far as we can see, upon the same conditions as that of urea. The relative amounts, however, vary considerably for reasons as yet unexplained. It is believed that certain diseases, rheumatism, and especially gout, are dependent upon the presence of an excess of uric acid in the system, and for this reason the presence of urates in large amounts is often an important point in diagnosis.

LABORATORY EXPERIMENTS.

UREA AND URIC ACID.

I. Urea. (*a*) *Quantitative Determination.*—Test at least three samples of urine, quantitatively, for urea by the hypobromite of soda method, as follows:

1st. *Doremus's Apparatus.*— Fill the apparatus full of the hypobromite solution. Then fill the small pipette up to

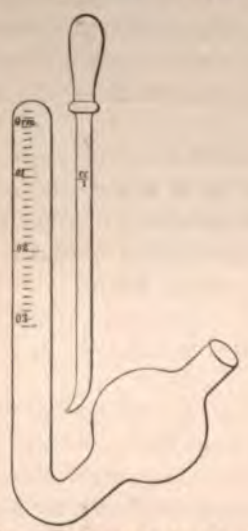


FIG. 15.—DOREMUS'S APPARATUS.



FIG. 16.—MARSHALL'S APPARATUS.

the mark with urine, place the end of it well under the upright tube of the apparatus, and force in the urine slowly and carefully. Take care that all the urine enters the hypobromite solution without any air being forced in at the same time, and that all the gas evolved remains in the graduated tube. When the effervescence has ceased, notice where the liquid stands on the scale, and read off the percentage of urea.



2d. *Marshall's Apparatus.*—Fill the apparatus full of the hypobromite solution. Then fill the long bent pipette full of urine, and allow, according to the concentration of the urine, 1, 2, or 3 c.c. of urine to flow gently in under the graduated tube. Three c.c. will be found sufficient even for quite dilute urines. Care must be taken in every case to admit the urine so slowly that no gas can escape from the apparatus. When the effervescence has ceased, insert the side funnel-tube, and fill it carefully with hypobromite solution until the level of the liquid inside and outside the measuring-tube is the same. Then calculate the percentage of urea by the rule, "The number of c.c. of nitrogen gas corresponding to 1 c.c. of the urine, multiplied by the factor 0.269, will equal the percentage of urea in the liquid."

After these determinations have been made and before proceeding with the rest of the lesson, rinse out the apparatus, return to the bottle all the unused hypobromite solution, and pour the rest down the sink. In case, also, any of the solution has been spilt on the desk, neutralize it with a little acid, and then sponge it off. Otherwise the fumes of the bromine will injure the microscopes.

(*b*) *Qualitative Test.*—Make the following tests on the sample of concentrated urine.

1st. *Nitrate of Urea.*—Lay a thread from a towel or handkerchief on a slide; on it put two or three drops of the liquid; lay on a cover-glass, and under this run a drop of HNO_3 conc. Examine under the microscope with a low power, and notice the rapid formation of large, flat, rhombic crystals of nitrate of urea.

2d. Place some of the liquid in two watch-glasses.

To the first add the oxalic acid dissolved in a drop or two of water. Notice, under the low power of the microscope, the formation of large, rhombic crystals of oxalate of urea.

To the second add a couple of drops of a concentrated solution of NaCl. Warm the watch-glass on a water bath

till it is nearly dry; then set it under the low power of the microscope, and notice the formation of flat crystals of sodium chloride urea.

3d. *Mercuric Nitrate Test.*—Add a little mercuric nitrate to a little of the liquid in a test-tube, and notice the dense white ppt. that results. Examine a little of this under the microscope.

II. Uric Acid.—(*a*) *Preparation.*—Boil some snake's excrement with a little water, and filter the hot solution into a small beaker containing one or two drops of HNO_3 dil. Notice the rapid separation of white crystals of uric acid. Examine these carefully under the microscope.

Also, from the small beaker of acidified urine set aside from the last lesson take a drop containing some of the dark-colored sediment, and examine under the microscope. Let the drop slowly evaporate on the slide, and notice the peculiar forms assumed by the uric acid.

(*b*) *Tests.*—Test the uric acid in both the above solutions as follows:

The Murexide Test.—Place some of the solution in a watch-glass, evaporate it to dryness, cover the residue with a drop or two of HNO_3 conc., and then evaporate to dryness on a water-bath. Let it cool, and then add a drop of very dilute NH_4OH. Notice the red color, due to the formation of "murexide."



Fig. 1. Urea from Water Solution, x 25.



Fig. 2. Urea Nitrate, x 75.



Fig. 3. Urea Oxalate, x 75.



Fig. 4. Urea Sodium Chloride, x 75.



Fig. 5. Uric Acid from Acid Urine, x 25.



Fig 6. Uric Acid from Snake's Excrement, x 200.

C. E. P. del.

LESSON XXIV.

ALBUMIN IN THE URINE.

Of the various proteids and albuminoids mentioned already, mucin is the only one that regularly occurs, at least in any appreciable quantity, in normal urine. Under abnormal conditions, however, the urine has been found to contain serum-albumin, paraglobulin, fibrin, albumose, peptone and egg-albumin.

Of these serum-albumin is by far the most important. It occurs in urine, usually mixed with more or less paraglobulin, in very small amounts, hardly ever over 1 or 2% by weight, and in most cases may be considered as an indication of some congestion or inflammation of the kidneys. This is true even when the amount of albumin passed in this way is exceedingly minute, and hence it is of the greatest importance to be able to recognize even the faintest traces. It must not, however, be forgotten that in some individuals very slight causes, such as a cold or similar indisposition, irregularities in diet or exercise, etc., are sufficient to produce a temporary, and in most cases a harmless, albuminuria.

It is also possible to have albumin in the urine derived from other sources than the kidneys, in which case it is of much less significance. It may come, indeed, from any part of the genito-urinary tract. In almost every doubtful case its origin may be determined by the aid of the microscope, for if the albumin comes from the kidney it is usually associated with casts; while if it comes from the bladder, urethra, or other parts, it is usually accompanied with pus, blood, or epithelial cells.

Tests for Albumin.—The best tests for albumin are those already specially described in Lesson XIX., where they were made upon diluted serum. All of these tests react with paraglobulin as well as with albumin, and the picric-acid tests also responds to albumose or peptone.

Of these tests the ring test with nitric acid, often known as Heller's test, will usually be found the most satisfactory. When carefully made it is exceedingly delicate. If, however, the urine is very concentrated, it may happen that a precipitate will be formed of acid urates or of uric acid. This ring differs from the ring produced by albumin, first, by being usually more or less brown in color, and secondly, by forming not at the exact juncture of the two liquids, but distinctly above it. The precipitate can only occur when the urine is very concentrated, and is much less liable to occur when the test is made with diluted instead of concentrated nitric acid. It can be obviated by previously diluting the urine with two or three parts of water.

A white ring is also formed by nitric acid, if either turpentine or balsam of copaiba are being taken in any quantities. These drugs can readily be detected in the urine by the smell, and the precipitate, which consists of resinous bodies, dissolves readily in alcohol.

This test is so consistent in its results that it is often used for a more or less approximate quantitative estimation. Without going into great details, it may be simply stated that a distinct cloud will form in two or three minutes in urine containing less than $\frac{1}{100}$ of 1% of albumin. The cloud will form at once with about $\frac{1}{10}$ of 1%, although it will be pretty faint, and must be seen against a dark background. From that point up to about $\frac{1}{2}$%, the ring will be more or less distinctly granular in appearance, and can be seen without a dark background, while above that the albumin settles in flocks and lumps.

The picric-acid test is a very good one also, and upon it is based Esbach's method for determining the quantity of albumin present by means of an "albuminometer." This is a tube with a series of fine graduations at the bottom, and with two marks on the side, noting the points to which it is to be filled, first with urine, and then with the test liquid, an aqueous solution containing 10 gms. picric acid and 20 gms. citric acid to the litre. The urine and the test liquid are carefully mixed together in the tube and allowed to stand for twenty-four hours, when the albumin will be settled to the bottom, in the form of a yellowish-white precipitate. Its quantity can be estimated according to the

graduation at which the precipitate stands. The results with this apparatus are only approximate.

Peptonuria.—Of late years the presence in urine of peptone, probably mixed with more or less albumose, has been frequently noticed, especially in connection with diseases where large amounts of pus are formed and broken down in the body. Thus it occurs in the later, softening and resolving stages of pneumonia, in purulent meningitis, pleurisy, phthisis, and in acute articular rheumatism. Peptone is also found in severe scurvy, and, normally, in the urine of women after childbirth.

To determine the presence of peptone, a considerable quantity of the urine is usually taken, and freed, first from mucin by treatment with neutral plumbic acetate, and then from any albumin that may be present by means of acetic acid and potassic ferrocyanide. The peptone can then be precipitated by a mixture of strong acetic acid and phospho-tungstic acid.

LABORATORY EXPERIMENTS.

ALBUMIN IN URINE.

In this lesson there will be provided numerous specimens of urine from patients suffering with different varieties of kidney disease. It is expected that the student will keep careful records of the general properties of these urines (*v.* Lesson XXII.), as well as examine them carefully by the following tests. For details of these albumin tests see Lesson XIX.

I. *Acetic-Acid and Heat Test.*—Fill a test-tube nearly full of urine. If the urine is alkaline, add 2 or 3 drops of acetic acid until it is just acid to test paper. If the urine is acid already, do not add anything. Then boil the top part of the liquid, and examine it carefully against a dark background. If the liquid, where it was heated, looks at all turbid, let it cool a minute or two, and then add a drop or two of HNO_3 dil. If the turbidity remains or is increased = Albumin.

II. *Ferrocyanide Test.*

III. *Nitric-Acid Ring Test.*

IV. *Picric-Acid Test.*

Make these last tests exactly as described in Lesson XIX.

Before each of these four tests, the urines, if not perfectly clear must be filtered.

LESSON XXV.

GLUCOSE IN URINE.

The urine not infrequently contains, both in health and disease, several representatives of the carbohydrate group, as, for instance, lactose in the urine of nursing women, and invert-sugar after rapidly digesting large quantities of cane sugar. These substances, however, possess little interest for us compared to the presence of dextro-glucose or dextrose.

Glycosuria.—Dextrose is often, if not always, present in perfectly normal urine, although in amounts altogether too small to be detected by the ordinary tests. It is occasionally found in much larger quantities as the result of temporary conditions, sometimes physiological, as, for instance, after taking an excess of readily absorbed carbohydrate food, and, more generally, in the course of certain diseases, such as cholera, meningitis, and liver disease, or as an effect of certain poisons, such as, for instance, carbonic oxide.

When, however, it is persistently present in the urine, it is a distinguishing mark of the disease known as diabetes mellitus, a disease characterized at the same time by an increase, often very great indeed, in the amount of urine excreted.

The urine from these patients is usually very light in color, on account of the great dilution of the pigments, and at the same time is unusually heavy on account of the presence of the glucose. The percentage of the urea is not as low as might be expected with such a great flow of urine, for along with the excessive excretion of carbohydrate matter there is at the same time a considerable increase in the total amount of urea excreted. The mere fact of a urine being light in color and at the same time having a specific gravity of over 1.025 or 1.030 is a strong indication of the presence of glucose.

Tests for Glucose in Urine.—(*a*) *Qualitative.*— The different tests for glucose have all been carefully described and explained in Lesson II., and the student is referred to that lesson for full details

The Moore's test, as already mentioned, is not as satisfactory with urine as with clear solutions of glucose, because the color of the urine tends to conceal the result.

The bismuth subnitrate test is not very satisfactory, for it does not always react promptly, even when glucose is present, while it is liable to react when other reducing compounds, drugs and the like, are present in the urine. It will be remembered that the Nylander's solution, as in Lesson X., reacts slightly with albumin as well as with glucose.

The picric-acid and potash test is valuable for practical work, because by it we can examine for albumin as well as for glucose in the same test-tube. It must not be forgotten, however, that when no glucose is present the color of the mixture is darkened somewhat, not only by the action of the potash upon the picric acid, but also by the presence in the urine of small quantities of creatinin and other reducing bodies.

The Trommer's and Fehling's tests are in many respects the most satisfactory. Unfortunately, however, many substances which occur not infrequently in both normal and pathological urines are able to reduce cupric hydrate, even when no glucose is present. Thus uric acid, creatin, mucin, urobilin, the bile pigments and similar substances, as well as compounds derived from the administration of balsam, copaiba, salicylic acid, glycerin and some less common medicines, react like glucose only, in general, with very much less energy.

For this reason the phenyl-hydrazin test is sometimes extremely important in cases where the other tests give dubious results. The reaction, if the directions on pages 24 and 25 are followed implicitly, can be obtained with certainty every time. No substance besides glucose will give these particular crystals, and only a carbohydrate will produce crystals at all under such circumstances. The appearance of the crystals is shown in Plate I., at the end of Lesson V.

Glycosuric Acid.—It is interesting in this connection to refer

to a substance, carefully isolated by Dr. John Marshall,* and called by him glycosuric acid, which has been more than once found in the urine of healthy individuals. In Dr. Marshall's case, the urine reacted excellently with both Trommer's and Fehling's tests, and was supposed to contain over 8% of glucose. The reduction, however, was all due to the presence of an organic acid, which crystallized in tetragonal prisms, was soluble in water and alcohol, formed salts with bases, and gave a brown color when heated with potash. It did not react with either picric acid or bismuth, nor did it undergo alcoholic fermentation.

(*b*) *Quantitative.*—*Fehling's Test.*—In spite of its disadvantages, the Fehling's test is still the most satisfactory quantitative method of examining urines for glucose. The urine must always be diluted ten times with water, before making the test, and even then must be added very slowly and carefully, because otherwise some of the reducing bodies in it are quite liable to bring down the copper in a greenish-yellow deposit, which does not become red even on continued boiling, and which completely spoils the reaction. If this happens, the best thing to do is to wash the flask out with water and a little acid, and begin the test over again with more care. If, however, the reaction once gets started right, it will run along smoothly, and will give as sharp an end reaction as when it is made with pure solutions of glucose. Still, samples of urine are met with, occasionally, so full of abnormal products that it is impossible, even with every precaution, to make this test satisfactorily.

Fermentation Test.—The fermentation method of analyzing urine for glucose is quite largely used by practitioners, on account of its simplicity. Besides determining the amount of sugar from the loss in specific gravity after the urine has been fermented, the carbon dioxide that is evolved is often measured, and the quantity of glucose calculated accordingly.

Unfortunately, however, this method presents many disadvantages. It is not very delicate, and indeed it does not react at all with less than about 0.5% of glucose. Nor is it particularly accurate, for the thorough conversion of the glucose into alcohol depends on many outside circumstances, such as the condition of

* Med. News, January 8th, 1887.

the yeast, the absence of other germs, the temperature and duration of the fermentation, and the like; while the presence of various compounds in the urine, especially those resulting from the external or internal administration of antiseptic drugs, such as mercuric chloride, iodoform, salicylic acid, quinine, and others, may stop the fermentation altogether.

LABORATORY EXPERIMENTS.

GLUCOSE IN URINE.

Examine the samples of diabetic urines with care, and make records of their general properties, and of the presence or absence of albumin, according to the directions given in Lessons XXII. and XXIII. Also examine at least three different specimens for glucose by the following tests, according to the directions given in Lesson II.

I. **Qualitative Tests.**

1st. *Moore's Test.*

2d. *Phenyl-Hydrazin Test.*—This test is extremely important, and the crystals should be made and recognized by each student.

3d. *Bismuth-Subnitrate Test.* (*Nylander's Test*).

4th. *Picric Acid and Potash Test.*—Add the urine to some picric acid in a test-tube, and notice the presence or absence of albumin. Then add an excess of KOH, and boil. Notice the deep brown or black color resulting from the presence of glucose.

5th. *Trommer's Test.*

6th. *Fehling's Test.*

II. **Quantitative Tests.**

1st. *Fehling's Test.*—Take with a pipette 5 c.c. of the $CuSO_4$, and mix it in a flask with the same amount of "rochelle and soda." Fill the flask one-third full of water, add a piece of pumice stone, and boil. While this is heating, dilute the urine ten times with water by placing 5 c.c. of urine in the 50-c.c. flask, and filling this to the mark with water. With this diluted urine fill the burette

to the 0 mark, and when the Fehling's solution is boiling add to it, drop by drop, some of the liquid from the burette. Every minute or two stop boiling, let the ppt. (which should be red) settle, and notice if the color has disappeared from the mixture in the flask. If it still shows a bluish tinge, boil again, add a few drops more of the diluted urine, and examine the color once more. The end of the reaction will be shown not only by the absence of this blue color, but also by the color of the ppt. changing from purple or dark-red to a bright vermilion.

When the blue color has entirely disappeared, calculate the percentage of glucose in the original urine according to the following rule: "The percentage of glucose will be equivalent to 50 divided by the number of c.c. of diluted urine used."

2d. *Fermentation Test.*—Mix with a sample of diabetic urine, whose percentage of glucose and whose specific gravity you have already carefully determined and noted, a little yeast which has been thoroughly washed upon a filter. Place the mixture in a small beaker and cover it with a plate of glass or another large inverted beaker, to avoid evaporation. At the end of twenty-four hours or later, if the fermentation has not yet ceased, pass the mixture rapidly through a filter and take the specific gravity of the filtered fermented urine. A difference of one degree in the specific gravity of the urine, before and after fermentation, is supposed to correspond to between $\frac{1}{4}$ and $\frac{1}{8}$ of 1% of glucose, or to about one grain of glucose to the fluid ounce. See how nearly the percentage obtained by this test agrees with that previously obtained by the Fehling's test.

PART IX.

MICROSCOPICAL EXAMINATION OF THE URINE.

THE MICROSCOPICAL EXAMINATION OF URINE.

INTRODUCTION.

FOR clinical purposes the results obtained by examining a sample of urine under the microscope are of even more importance than those obtained by the chemical tests hitherto described. The sediment which is to be submitted for examination is usually obtained by letting the urine settle for some hours in a conical glass, and then obtaining a few drops from the bottom, either directly, by means of a pipette or glass tube, or after carefully pouring off the top liquid. The practice has recently been introduced of putting the urine, contained in a test-tube or long slender flask, into a centrifugal machine, which, on being rapidly rotated, swings all the suspended matter to the bottom in a very few minutes.



FIG. 17.—CENTRIFUGAL SEPARATOR FOR URINE SEDIMENTS.*

The urine thus concentrated should be spread out in a thin film on a glass slide, no cover-glass being necessary or, indeed, advisable, and carefully examined under first the low power and then the high power of the microscope. The objective used for the latter purpose need rarely be of greater magnifying power than a one-sixth (inch) objective, while a two-thirds objective will do very well for the preliminary examination. It should be remembered, as a general rule, that an object that is being studied under the microscope should, if possible, be kept moist.

In the course of the following lessons the student is expected to make himself familiar with the principal forms and varieties

* Can be obtained from J. T. Dougherty, 348 W. 59th St., New York.

of urinary deposits. The specimens will be given out to him, as nearly as possible in the order described, by the demonstrator, who will discuss, at the same time, the principal features to be noticed. The student is strongly advised to make careful drawings of the different crystals and deposits, and to compare them with the illustrations, not only in the text, but also, wherever possible, in other illustrated works upon urine, such as Tyson's "Practical Examination of Urine," Ultzmann and Hoffmann's "Atlas des Harnsedimente," Peyer's "Atlas of Clinical Microscopy," and others.

LESSON XXVI.

SEDIMENTS IN ACID URINE.

URIC ACID, URATES, CALCIUM OXALATE, ETC.

The urine as passed usually has an acid reaction, and continues acid for some hours until the ammoniacal fermentation has fairly set in. During this period there may be deposited any of the following compounds:

(*a*) Uric acid.

(*b*) Acid urates of sodium, potassium, ammonium and calcium;

(*c*) Calcium oxalate;

and more rarely—

(*d*) Hippuric acid;

(*e*) Calcium sulphate.

Uric Acid.—This occurs, usually as a red sediment readily seen by the naked eye, in urines that are strongly acid. The crystals are generally quite large, and often form little bunches or concretions, which appear like grains of red sand or gravel, at the bottom or along the sides of the glass containing the urine.

Under the microscope they present very varied forms, ranging from the characteristic whetstone shape to long pointed prisms. They are often united together, forming rosettes and other figures which are sometimes quite beautiful (Plates VI. and VII.).

The crystals can be readily recognized by their color, which in the natural urine is always red or yellow, but, if mineral acid has been added, may be either brown or purple. The crystals dissolve readily in alkalies, and can be reprecipitated from this solution, although slowly, by acidifying. They can also be separated by filtering, and tested by the murexide test, as in Lesson XXIII.

The presence of uric-acid crystals, after the urine has stood for some time, is not always a matter of much importance. When,

however, the urine is so full of uric acid that the crystals separate almost as soon as it is passed, there may be danger of their precipitating in the bladder, and thus giving rise to irritation of the bladder, or even to the formation of uric-acid calculi.

Acid Urates.—These compounds, although always present in urine, rarely form deposits unless the urine is quite concentrated and is chilled. An excess of these salts, as well as of uric acid itself, is quite common in the urine of fever patients, and also of patients subject to either gout or rheumatism. Generally, however, the alarming-looking sediments which are so often met with in cold weather when the urine has been chilled have absolutely no clinical significance. They are readily recognized by dissolving in alkalies or on the application of heat.

The sediment formed by the urates is almost always amorphous or granular. It ranges in color from a yellowish-white to a quite bright red, the so-called brick-dust sediment. It is principally composed of sodium urate, which also very rarely occurs in a crystalline form as radiating colorless masses or as star-shaped groups of colorless prisms.

Among other constituents of the amorphous urate sediment may be mentioned the acid urates of potassium and also, it is supposed, of calcium. When the ammoniacal fermentation has commenced, even though the urine is still acid, it is often possible to detect the brown, spherical masses of urate of ammonium, described in the next lesson.

Calcium Oxalate.—This salt, which has already been studied in Lesson XIV., occurs not infrequently in both normal and pathological urine, and is especially common after a diet of rhubarb, asparagus, and other vegetables. It sometimes separates from the urine while still in the bladder, giving rise not only to irritation and inflammation of that organ, but also to the well-known oxalate of lime or mulberry calculus.

The crystals, as they occur in urine, belong to two distinct classes. The commonest and most characteristic form is that of octahedra (Plate III.), with high refracting powers, which are at once recognized, under the microscope, as colorless squares or rectangles with diagonal lines.

Besides these, we sometimes find what are known as "hour-



A

Uric acid crystals.



B

Sediment in acid urine.

glass" crystals, oval-shaped bodies, not much unlike a side view of a large colorless red blood-cell. The depression in the sides may be more and more pronounced until it gives rise to the "dumb-bell" form, where the crystal consists of two little spheres united by a larger or smaller band.

These crystals are much smaller in size than those of uric acid, and can rarely be recognized without the aid of the one-sixth objective. They often make their appearance soon after the urine is passed; but as they are soluble in strongly acid urine, they are sometimes only precipitated after the alkaline fermentation has commenced. They do not dissolve in alkalies, and hence will still be found after the urine has become strongly ammoniacal.



FIG. 18.—CRYSTALS OF CALCIUM OXALATE. × 350.

Hippuric Acid.—This compound is excreted by herbivorous animals in large amounts, but it is present in human urine only in small quantities, and hence is but rarely found as a sediment. Occasionally, however, as for instance after taking benzoic acid or after feeding on certain kinds of fruit, crystals of hippuric acid are found in the urine. It has no clinical significance.

The crystals vary considerably in size. They occur usually as colorless prisms with sharply defined ends, or, more rarely, as colorless needles. They can be distinguished from uric acid, which in other chemical properties they somewhat resemble, by not reacting with the murexide test.

Calcium Sulphate.—The crystals of this compound have already been examined in Lesson XIV. They occur occasionally in the urine in the form of radiating needles (Plate II.), but are of no particular importance.

LESSON XXVII.

SEDIMENTS IN ALKALINE URINE.

After the urine has stood for some hours at a moderate temperature it becomes alkaline, from the formation of ammonium carbonate, and then a new series of deposits make their appearance. These are all characterized by being soluble in dilute acids and by not dissolving when warmed.

They are mostly composed of the amorphous earthy phosphates, which are regularly precipitated in urine that is alkaline when passed, as well as in urine that has undergone fermentation. Besides these we have, in crystalline form:

(*a*) Triple phosphates.

(*b*) Ammonium urate.

(*c*) Calcium phosphate.

(*d*) Calcium carbonate.

There may also occur, usually under pathological conditions, the rare deposits of leucin, tyrosin, and cystin.

Triple Phosphate—$MgNH_4PO_4 . 6H_2O$.—This compound has already been studied in Lesson XIV. It occurs regularly as a deposit in fermenting ammoniacal urine, and is only to be considered as abnormal when it is present in the urine as it leaves the bladder.

The feathery shaped crystals (Plate III.), which are usually found when testing solutions for the presence of Mg, occur but rarely in urine. Instead of these, we usually find the so-called "coffin-lid" crystals, colorless rectangular prisms with bevelled faces. They range in size from extremely minute crystals up to great transparent masses, which have but little definition, but are still recognized as triple phosphate by their color, the angles which they show, and their presence in an ammoniacal medium.

Ammonium Urate.—Mixed with the triple phosphate we almost always find characteristic yellow or brown spheres, gener-

ally with spikes and projections adhering to them, which we can recognize as ammonium urate. They vary in shape very much, but can always be distinguished by their color, their appearance, and their surroundings. For the typical appearance of urine undergoing alkaline fermentation, see Plate VI.

Calcium Phosphate—$Ca_3(PO_4)_2$, or possibly $CaHPO_4$.—This usually forms amorphous deposits, but now and then occurs in the form of colorless, wedge-shaped, prismatic crystals, which vary considerably in size and shape, and often form stars, with the small ends of the wedges pointing toward the centre. They are found not only in alkaline, but also in slightly acid urines which are very nearly neutral. They have no clinical significance.



FIG. 19.—CRYSTALS OF CALCIUM PHOSPHATE. × 150.

Calcium Carbonate.—This rarely occurs in human urine, and then, usually, in the form of little grains and spheres, which evolve CO_2 when treated with a little acetic acid. They are of no special importance.

In all samples of urine undergoing alkaline fermentation, it is easy to recognize immense quantities of bacteria, as well as, frequently, of yeast and mould plants.

Leucin and Tyrosin.—These substances, already described in Lesson XXI., occur occasionally in the urine in cases of acute disease of the liver. They are usually accompanied by considerable amounts of bile pigments, which can be readily tested for by Gmélin's reaction.

They are usually recognized in urine by the characteristic crystals (see page 226), which, if not present in the natural urine, will generally deposit, if present, when a few drops of the urine are gently evaporated on a slide. The leucin, as before mentioned, occurs in little yellow or brownish spheres, much like little drops of oil; while the tyrosin forms sheaves and tufts of fine, delicate, colorless needles.

Cystin.—This substance occurs rarely in the urine, as a result of some unknown changes in metabolism. It is usually found as a whitish deposit, consisting of small, regular, six-sided plates. It also occurs in irregular masses, and not infrequently gives rise in the bladder to cystin calculi.



FIG. 30.—CRYSTALS OF CYSTIN. (FREY.)

Cystin differs from most of the other organic compounds of the body by containing large quantities, some 26%, of sulphur. Its composition, as given by Hoppe-Seyler, is $C_3H_7NSO_2$. It is insoluble in water and in solutions of ammonium carbonate, but dissolves in acids and in caustic alkalies.



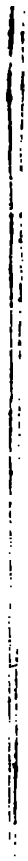
A

1. Calcium phosphate. 2. Triple phosphate.



B

Urine undergoing alkaline fermentation.
Ammonium urate; triple phosphate; bacteria.





LESSON XXVIII.

CASTS.

These bodies, the so-called casts of the uriniferous tubules, are of very great clinical importance, and, when carefully studied, often enable the observer not only to diagnose a case of kidney disease, but actually to follow its course, and to keep track of the condition of the kidney itself. For this reason the student is advised to examine these specimens with the utmost care, and to become thoroughly familiar with the appearance and properties of the different varieties exhibited.

Formation.—As already stated, in perfectly normal conditions no albuminous matter passes from the kidney in the urine. When, however, from one cause or another, some of the proteids of the blood are allowed by the epithelial cells of the lining to pass into the tubules, the urine is not only made albuminous, but is liable to contain little plugs of albuminous matter that has been coagulated on the way. This coagulation occurs as the albuminous matter is passing along the long and tortuous course of the tubules, and the little clots or plugs thus formed stop up the tubule more or less completely, and probably prevent it, for the time being, from delivering any urine. When, however, the back pressure of the fluid behind the plug reaches a certain point, the little clot of albuminous matter is forced down through the windings and folds of the tubule, and finally is driven out into the urine. These little clots are what we designate as casts, for they may be considered as casts of the parts of the tubule where they have been formed, made out of coagulated proteid matter.

General Appearance.—The casts thus formed are minute, cylindrical masses with parallel, though often much curved and twisted, sides. Generally speaking, they have much the shape of a finger. It is important to remember that the material from which a cast is made is soft and tough, and has been rubbed and

rolled around a good deal. Hence, one end, at least, is almost invariably rounded off. They need a magnifying power of at least the one-sixth objective for proper definition, although they can often be recognized, especially if dark colored, by a much lower power. It is advisable, particularly when looking for "hyaline" casts, to keep the field of the microscope rather dark.

Varieties.—The casts, formed as just described, will naturally look like little particles of coagulated proteid; *i.e.*, they will be comparatively colorless and transparent, and will show but little signs of any structure. These are called hyaline (glassy) or, when more opaque, waxy casts, and generally possess no further significance than the presence in the urine of a little albumin. It has even been claimed that some varieties of hyaline casts do not



FIG. 21.—CASTS. *a*, Hyaline; *b*, waxy; *c*, hyaline and granular; *d*, hyaline and epithelial; *e*, hyaline and blood; *f*, hyaline and pus.

come from the proteids of the blood itself, but are secreted by the epithelial cells. When, however, the disorder in the kidneys has progressed so far that the lining itself is disintegrating, not only do the casts become more abundant, but the little plugs of albuminous matter, as they work down through the tubules, pick up parts of the epithelial lining. Hence we may find, adhering to the casts, either more or less granular matter from the disintegration of the cells, or even some of the epithelial cells themselves, which have been detached bodily from the walls of the tubules. The latter we usually call epithelial casts, and the former, according to the structure of the grains, either fine or coarse granular casts.

Besides the casts which have as a basis the hyaline or waxy clots of albuminous matter, it is probable that many casts are formed by the filling up of the tubules by the products of inflam-

mation. Thus we have granular casts, both fine and coarse, where the mass is entirely composed of granules; and these casts, like the hyaline, may have epithelial, blood, or pus cells adhering to them, when they are named (granular) epithelial, blood, and pus casts respectively. Rarely we find fatty casts, where the minute, shiny globules of fat, either free or in epithelial cells, are imbedded in granular or hyaline casts.

When examining these casts under the microscope, it will be noticed that the different varieties all merge into one another, and that in almost every case they must be described as hyaline and granular, or hyaline and epithelial, or granular and pus casts, instead of being described by one name only.

Clinical Importance.—It is hardly our place to speak in detail about the relative significance of each of these varieties. In



FIG. 22.—CASTS. *a*, Fine granular; *b*, coarse granular; *c*, epithelial; *d*, blood; *e*, pus; *f*, fatty.

general, however, it is usually agreed that the least alarming are the hyaline and, probably, the waxy casts. As the disease increases in intensity the granular casts appear in greater abundance; while in the acute stages of inflammation the casts are found to carry more and more epithelial, blood, and pus cells. The latter indicate a purulent inflammation of the kidneys, and, with the blood casts, are of great importance in diagnosis, as showing that the pus cells and blood found in a sample of urine actually come from the kidneys, and not from some other part of the genito-urinary tract.

The casts are not much heavier than water, and hence settle rather slowly in the urine, as it stands in a conical glass. The usual rule is to add some antiseptic, such as thymol or carbolic acid, to the urine, and to let it settle for at least twelve hours before examining the sediment. The casts can be separated much more rapidly and perfectly by centrifugal action.

Owing to their great importance, any sample of suspected urine should always be examined for casts on at least two or three different slides, before concluding that they are absent. It generally saves much time to run over the sample first with an objective of low power, and then to focus carefully with the higher magnifying power on any object which looks suspicious.

of hæmoglobin in the urine by means of either the hæmin or the guaiacum tests of Lesson XX. The latter is extremely delicate and can be applied to urine in a test-tube, without the necessity of staining a piece of paper with it. But unfortunately it reacts with so many other substances—pus, epithelial cells, spermatic fluid, and the like—that it is of value only as a negative, and not as a positive test. It is far better to evaporate a little of the urine to dryness in a watch-glass, and to make the hæmin test on some of the residue.

Blood, if present in the urine, may come from any part of the genito-urinary passages. If it comes from the kidney, in which case it is an extremely grave symptom, it will usually be accompanied with some blood casts. It is normally present in female urine passed at the menstrual period.

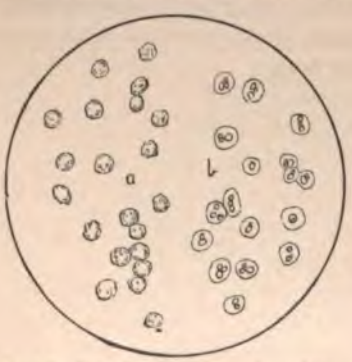


FIG. 24.—PUS CELLS. *a*, Natural condition; *b*, after the addition of acetic acid.

Pus Cells.— These may be briefly described as broken-down white blood-cells which have been killed while engaged in combating some inflammatory process.

Properties. — They are readily distinguished from blood cells by being distinctly granular in structure, and having a rather irregular outline. They are somewhat larger than the blood-cells, and are quite colorless under the microscope. When present in great abundance, they may give the urine a white or creamy appearance.

When pus cells are treated with a little acetic acid, they lose their granular appearance and their nuclei become distinctly visible. The addition of potassic hydrate, on the other hand, causes them to dissolve to a gelatinous mass. This also occurs when the pus cells are allowed to stand for a while in alkaline urine.

Derivation.—These cells may occur in the urine from inflammation of any part of the genito-urinary tract. When they come

from the kidney, they indicate a purulent inflammation of that organ, and are usually accompanied with pus casts and with considerable amount of albumin.

When they come from the bladder, in cystitis, the urine is usually undergoing decomposition and not infrequently is alkaline enough to convert the cells into a slimy mass much like mucus, sometimes before they leave the bladder. In female urine pus cells are frequently present, although usually in but trifling amounts, from slight chronic affections of the vagina and sometimes of the neck of the uterus, as well as in more acute disorders.

Urethritis.—In male urine pus very frequently is present from inflammations of the urethra, both acute and chronic, and in both cases it is a very important means of diagnosis. In acute attacks it is of great importance to limit the inflammation to the anterior portion of the urethra; and as long as this is the case, the urine that is first passed will contain all the pus and other products of inflammation. But when, from one cause or another, the disease has passed the triangular ligament, and reached the posterior part of the urethra, some of the pus constantly runs back into the bladder and there mixes with the urine. Hence in all cases of posterior urethritis, as well as of the prostatitis and even cystitis which are then so liable to follow, the last urine that leaves the bladder will still contain some pus cells. For this reason, in all cases of urethritis it is advisable to have the urine that is to be examined passed first into one, and then, after the urethra has been cleansed by the flow of liquid, into a second receptacle. These two samples should then be examined for pus cells.

Another very important point, which can best be settled by the examination of the urine for pus cells, is whether a case of urethritis has been thoroughly healed, or whether there is still some chronic inflammation. In quite a large percentage of cases, even where the attack has been slight and without complications, and has apparently been completely healed, there still remains a little inflammation at some point or another, either behind a stricture, or at some so-called granulating patch, or, very commonly, just behind the meatus. These spots often remain un-

healed for years, and all that time a patient may be in an infectious condition, or may, according to some authorities, be liable to a return of the acute symptoms. By examining the first urine that is passed, it is always possible, in such cases, to detect the so-called gonorrhœal threads, which are little strings of pus cells with generally some epithelial cells from the urethra, or even from the neck of the bladder, tied together with mucus.



FIG. 25.—GONORRHŒAL THREADS, WITH FEW SPERMATOZOA.

By careful examination of these threads, cleansed from urine, split open, dried on a slide, and stained with fuchsin, the presence of gonococci may often be detected in patients who for years have considered themselves, and have been considered, quite free from infection.

Epithelial Cells.—These occur in small quantities in all urines, but are specially abundant in certain diseased or abnormal conditions. They can be described as masses of protoplasm with single nuclei, and, usually, more or less granular in structure. They may come from any part of the genito-urinary tract, and different authors have tried to identify the various cells and state positively their origin. This, however, is rarely possible, even under the most favorable circumstances, and for all practical purposes it is best to divide them simply into round, columnar, and squamous cells.

Round Epithelial Cells.—These are very much like large pus cells, but differ from them by their size, and also by having only a single nucleus.

These round cells are usually supposed to come either from the pelvis of the kidney, or from the male urethra. Some of them may come from the bladder, and also, it is claimed, from parts of the uriniferous tubules. In the latter case they are usually associated with albumin and casts.

Columnar Cells.—These are of various shapes and sizes, and

generally come from small passages like the urethra and ureters, and, probably, the tubules of the kidney. It is claimed that some of them may also be derived from the kidney pelvis.

Squamous Cells.—These are the large, flat "pavement" epithelial cells which cover broad surfaces, and hence are usually derived from the vagina or the bladder. The vaginal cells are generally the largest and often come in sheets formed by several cells overlapping one another like the tiles on a roof. The bladder cells are generally smaller and thicker.

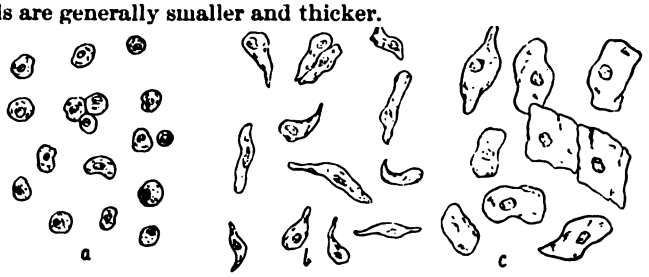


FIG. 26.—EPITHELIAL CELLS. *a*, Round; *b*, columnar; *c*, squamous.

All these cells are, usually, somewhat swollen and broken down by the action of the urine itself, especially if alkaline, and of bacteria. Interesting specimens containing these cells from various parts of the vagina and uterus, in all stages of decomposition, mixed with a little blood, can be obtained from women immediately after child-birth. As the uterus returns to its natural condition some of the vascular tissue, left behind after the expulsion of the placenta, undergoes fatty degeneration, and forms a discharge known as lochia, which passes off in the urine. In normal cases this diminishes quite rapidly, and finally ceases in the course of four or five days.

LESSON XXX.

SPERMATOZOA, MICROBES, FOREIGN BODIES.

Spermatozoa.—These are found not infrequently in the urine both in health and in disease.

They are exceedingly minute bodies with a head shaped something like a pear or an acorn, and with a long, slender, tapering tail. They cannot be seen with anything but a high-power objective.



FIG. 27. — HUMAN SPERMATOZOA, VERY HIGHLY MAGNIFIED. (FREY.)

Microbes.—These have already been quite carefully discussed in Lesson IV. In decomposing urine we frequently find examples of all three classes, the moulds, yeast plants, and bacteria, illustrations of which have been given in Plate I.

The mould plants are the least common, and occur usually after the urine has been standing for some time, excepting when glucose is present, when they appear in large quantities just after the alcoholic fermentation. The mycelium is the only part of the plant that develops in urine, and it is easily recognized by being in the form of slender strings, of greater or less length, composed of colorless, oval cells placed end to end.

The yeast fungi are present in small quantities in almost all decomposing urines, although they develop rapidly and in great abundance only in urines containing glucose. They consist of round or oval-shaped, nucleated, colorless cells, to whose sides some buds or small cells are usually attached.

The bacteria are invariably present in abundance, and can be easily recognized, with a high-power objective, in all decomposing urines. The micrococcus ureæ is perhaps the most important of the bacteria that are connected with the ammoniacal fermentation, but with it are associated bacteria of every size and shape.

These germs are in most cases non-pathogenic, and hence are not indicative of any disease, unless, as in cystitis, for instance, they cause the urine to decompose inside the body. It is, however, claimed by a large number of excellent authorities that ordinary bacteria, or even specific disease-germs, have frequently been met with in the fresh urine in certain diseases. Thus they have been found in acute nephritis, ulcerative endocarditis, erysipelas, and also in pneumonia, typhoid fever, glanders, and especially tuberculosis. In the latter case the bacilli may be detected by drying and staining, just as in sputa, and their presence may sometimes indicate the true nature of a kidney disease, occurring in tuberculous patients.

Foreign Bodies.—As a rule the power of at once distinguishing foreign bodies, such as particles of dirt or dust, air bubbles, threads, fibres, pieces of clothing, etc., which constantly occur in urine, can only be acquired by practice. There are, however, a few points about some of them which it may be well for the student to remember.



FIG. 28.—FIBRES. *a*, Wool; *b*, cotton; *c*, linen. × 25.

Air Bubbles. — When air bubbles are of large size, it is impossible to mistake them for anything else; but when very small, they are often mistaken by the inexperienced student for fat-globules and even for red blood-cells. When by themselves they are circular in shape and can always be distinguished by their very sharp, distinct, strongly refracting outlines, and by the total absence of any structure.

Threads and Fibres.—These are often found in samples of urine, derived either from clothing, or from towels and washing-cloths, or from the dust of carpeted rooms. They are not infrequently mistaken for casts, but can readily be distinguished, not only by their general appearance, but also, with great ease, by examining their ends. The cast is formed of a soft material, and

hence one or both of its extremities are always more or less rounded and smoothed off. But fibres always have either square ends or ends that are broken and jagged.

The commonest fibres met with in urine are of wool, cotton, or linen. The wool fibres are of animal origin, and are composed of cells whose edges, as seen in the figure, give the fibre a somewhat serrated structure, and thus, by the way, give it its felting properties. The cotton and linen fibres, on the other hand, consist of cellulose, and present no special structure. It will be noticed that the cotton threads have a curious twisted appearance which the others lack.



APPENDIX A.

TABLE OF WEIGHTS AND MEASURES.

ENGLISH WEIGHTS.

TROY WEIGHT.

Pound.	*Ounces.*	*Pennyweights.*	*Grains.*		*French Grammes.*
1	12	240	5,760	=	373.2419
	1	20	480	=	31.1035
		1	24	=	1.5552

APOTHECARIES' WEIGHT.

lb. *Pound.*	℥ *Ounces.*	ʒ *Drachms.*	℈ *Scruples.*	gr. *Grains.*		*French Grammes.*
1	12	96	288	5,760	=	373.2419
	1	8	24	480	=	31.1035
		1	3	60	=	3.8879
			1	20	=	1.2959
				1	=	0.0648

AVOIRDUPOIS WEIGHT.

Pound.	*Ounces.*	*Drachms.*	*Grains.*		*French Grammes.*
1	16	256	7,000	=	453.5926
	1	16	437.5	=	28.3495
		1	27.343	=	1.7718

METRIC MEASURES.

MEASURES OF LENGTH.

1 Millimetre	=	0.001 of a metre.		
1 Centimetre	=	0.010 of a metre.		
1 Decimetre	=	0.100 of a metre	=	about 4 inches.
1 Metre	=	**1.000 metre**	=	**39.37 inches.**
1 Decametre	=	10.000 metres.		
1 Hectometre	=	100.000 metres.		
1 Kilometre	=	1,000.000 metres	=	about ⅝ of a mile.
1 Myriametre	=	10,000.000 metres	=	about 6 1-5 miles.

MEASURES OF SURFACE.

1 Centiare	=	1 Square metre	=	about 1 1-5 square yards.
1 Are	=	**100 Square metres.**		
1 Hectare	=	10,000 Square metres	=	about 2½ acres.

MEASURES OF VOLUME.

1 Cubic Metre	=	1,000 Cubic decimetres.
	=	1,000 Litres, or one Kilolitre.
	=	1 Stere.

MEASURES OF CAPACITY.

1 Litre	=	1 cubic decimetre, or 1,000 cubic centimetres	=	**about 1 quart.**

MEASURES OF WEIGHT.

1 Milligramme	=	0.001 of a gramme	=	about 1-65 of a grain.
1 Centigramme	=	0.010 of a gramme.		
1 Decigramme	=	0.100 of a gramme.		
1 Gramme	=	**1.000 gramme**	=	**about 15½ grains.**
1 Decagramme	=	10.000 grammes.		
1 Hectogramme	=	100.000 grammes.		
1 Kilo(gramme)	=	1,000.000 grammes	=	about 2 1-5 lbs.
1 Tonneau	=	1.000.000 Kilos	=	about 1 ton.

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APPENDIX B.

ALPHABETICAL TABLE OF EQUIVALENT VALUES OF WEIGHTS AND MEASURES.

1 Are = 100 sq. metres	119.6 sq. yards.
1 Centiare = 1 sq. metre = 10,000 sq. centimetres	10.76 sq. feet.
1 Centimetre = 1-100 of a metre	0.3937 inch.
1 Cubic centimetre (of dist. water weighs 1 gm.)	.0610 cub. inch.
1 Cubic decimetre (same as 1 litre)	1.000 c.c.
1 " " of distilled water weighs 1,000 gms. or	1 kilogramme.
1 " " *in English* or imperial measure	0.8804 quart.
1 " " *in American* or wine measure	1.0567 quarts.
1 Cubic foot (1,728 cubic inches)	28,315.3119 cub. cent.
1 " " of water (at 62° F.) weighs	62.3210 lbs. Av.
1 Cubic inch	16.3861 cub. cent.
1 " " of water (at 62° F.) weighs	252.458 grains.
1 " " of water (at 60° F.) weighs	252.5 grains.
1 Cubic metre (1 stere) = 1,000,000 c.c. or	1,000 litres.
1 Fluid ounce, imperial = 28.4 c.c.	1.7329 cub. inches.
1 " " wine measure = 29.5 c. c.	1.8047 cub. inches.
1 " " imperial, of water (62° F.) weighs	437.5 grains.
1 " " wine measure, of water (60° F.) weighs	456.0 grains.
1 Foot	30.48 centimetres.
1 Gallon, imperial = 277.274 cubic inches	4.5435 litres.
1 " " of water weighs (62° F.) 10 lbs. or	70,000 grains.
1 Gallon, wine measure = 231 cubic inches	3.7852 litres.
1 " " " of water weighs (60° F.) 8.34 lbs. or	58,372.2 grains.
1 Gramme (weight of 1 c.c. of dist. water, 4° C.)	15.4323 grains.
1 Inch	2.54 centimetres.
1 Kilogramme (1,000 grammes)	2.2046 lbs. Av.
1 Litre (see Cubic decimetre).	
1 Metre (1 40-mill'th of earth's meridian) 3 ft. 3 in. $\frac{3}{8}$ in., nearly	39.3708 inches.
1 Pint, wine meas. = 16 fluid oz. = of water (60° F.) 7,296.5 gr	473.148 cub. cent.
1 " imperial = 20 fluid oz. = of water (62° F.) 8,750 gr	567.932 cub. cent.
1 Quart, wine measure = 32 fluid ounces	0.9463 litre.
1 " imperial 40 fluid ounces	1.1358 litres.
1 Ton avoirdupois (2,000 lbs.)	29,166$\frac{2}{3}$ oz. Troy.
1 Tonneau = 1,000,000 gms	1,000 kilos.

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APPENDIX C.

TABLE OF ATOMIC WEIGHTS.

(ISSUED DECEMBER 6TH, 1890.)

Revised for the Committee of Revision and Publication of the Pharmacopœia of the United States of America.

BY F. W. CLARKE,

Chief Chemist of the United States Geological Survey.

This table represents the latest and most trustworthy results, reduced to a uniform basis of comparison, with Oxygen = 16 as starting-point of the system. No decimal places representing large uncertainties are used. When values vary, with equal probability on both sides, the mean value is given in the table.

Name.	Symbol.	Atomic Weight.	Name.	Symbol.	Atomic Weight.
Aluminium	Al	27.	Molybdenum	Mo	96.
Antimony	Sb	120.	Nickel	Ni	58.7
Arsenic	As	75.	Nitrogen	N	14.03
Barium	Ba	137.	Osmium	Os	191.7
Bismuth	Bi	208.9	Oxygen[4]	O	16.
Boron	B	11.	Palladium	Pd	106.6
Bromine	Br	79.95	Phosphorus	P	31.
Cadmium	Cd	112.	Platinum	Pt	195.
Caesium	Cs	132.9	Potassium	K	39.11
Calcium	Ca	40.	Rhodium	Rh	103.5
Carbon	C	12.	Rubidium	Rb	85.5
Cerium	Ce	140.2	Ruthenium	Ru	101.6
Chlorine	Cl	35.45	Samarium	Sm	150.
Chromium	Cr	52.1	Scandium	Sc	44.
Cobalt	Co	59.	Selenium	Se	79.
Columbium[1]	Cb	94.	Silicon	Si	28.4
Copper	Cu	63.4	Silver	Ag	107.92
Didymium[2]	Di	142.3	Sodium	Na	23.05
Erbium	Er	166.3	Strontium	Sr	87.6
Fluorine	F	19.	Sulphur	S	32.06
Gallium	Ga	69.	Tantalum	Ta	182.6
Germanium	Ge	72.3	Tellurium	Te	125.
Glucinum[3]	Gl	9.	Terbium	Tb	159.5
Gold	Au	197.3	Thallium	Tl	204.18
Hydrogen	H	1.007	Thorium	Th	232.6
Indium	In	113.7	Tin	Sn	119.
Iodine	I	126.85	Titanium	Ti	48.
Iridium	Ir	193.1	Tungsten	W	184.
Iron	Fe	56.	Uranium	U	239.6
Lanthanum	La	138.2	Vanadium	V	51.4
Lead	Pb	206.95	Ytterbium	Yb	173.
Lithium	Li	7.02	Yttrium	Yt	89.1
Magnesium	Mg	24.3	Zinc	Zn	65.3
Manganese	Mn	55.	Zirconium	Zr	90.6
Mercury	Hg	200.			

[1] Has priority over Niobium.
[2] Now split into Neo- and Praseo-didmium
[3] Has priority over Beryllium.
[4] Standard or basis of the system.

APPENDIX D.

LIST OF REAGENT BOTTLES AND THEIR CONTENTS.

Issued to students of the College of Physicians and Surgeons, New York, for the course in MEDICAL AND PHYSIOLOGICAL CHEMISTRY.

On each desk.

Label.	Chemical Formula.	Number.	Size.	Remarks.
Hydrochloric acid conc.	HCl conc.	1	250 c.c. (8 oz.) N. M.	C. P.
Hydrochloric acid dil.	HCl dil.	2	" " "	1 vol. acid to 3 water.
Nitric acid conc.	HNO_3 conc.	3	" " "	C. P.
Nitric acid dil.	HNO_3 dil.	4	" " "	1 to 3.
Sulphuric acid conc.	H_2SO_4 conc.	5	" " "	C. P.; not filled.
Sulphuric acid dil.	H_2SO_4 dil.	6	" " "	1 to 3.
Hydrosulphuric acid.	H_2S.	7	" " "	Not filled.
Potassic hydrate.†	KOH	8	" " "	1 part KOH in 10 c.c. of solution.
Sodic carbonate.*	Na_2CO_3.	9	" " "	1 in 10.
Ammonic hydrate.*	NH_4OH.	10	" " "	1 vol. NH_4OH C. P. to 3 water.
Ammonic carbonate.*	$(NH_4)_2CO_3$.	11	" " "	1 gm. $(NH_4)_2CO_3$ and 1 c.c. NH_4OH in 5 c.c. solution.
Ammonic chloride.	NH_4Cl.	12	" " "	1 to 10.
Ammonic sulphide.	$(NH_4)_2S$.	13	" " "	Not filled.
Ammonic oxalate.	$(NH_4)_2C_2O_4$.	14	" " "	1 in 10.
Baric chloride.	$BaCl_2$.	15	" " "	1 in 10.
Hydro-disodic phosphate.*	Na_2HPO_4.	16	" " "	1 in 10.
Common alcohol.	C_2H_5OH.	Not numbered	" " "	95%.
Cupric sulphate.	$CuSO_4$.	" "	" " "	34.64 gms. $CuSO_4$ in 500 c.c.
Rochelle and soda.†	$KNaC_4H_4O_6$ + NaOH.	Not numbered†	" " "	157 gms. $KNaC_4H_4O_6$ + 68 gms. NaOH in 500 c.c.
Iodine.	I.	" "	125 c.c. (4 oz.) N. M.	1 gm. I and 1 gm. KI in 100 c.c.
Fuchsin.		" "	" " "	Strong alcoholic solution of fuchsin diluted with water till just transparent.
Argentic nitrate.	$AgNO_3$.	" "	" " "	1 in 20.
Platinic chloride.	$PtCl_4$.	" "	" " "	1 in 20.
Sodic chloride.	NaCl.	" "	125 c.c. (4 oz.) W. M.	Dry.

* Stopper greased with mixture of equal parts vaseline and paraffin.

† Rubber stopper.

For two desks.

Label.	Chemical Formula.	Number.	Size.	Remarks.
Common hydrochloric acid.	Common HCl.	Not numbered	500 c.c. (16 oz.) N. M.	Concentrated, not C. P.
Common sulphuric acid.	Common H_2SO_4.	" "	" " "	" "
Potassic ferrocyanide.	K_4FeCy_6.	17.....	200 c.c. (8 oz.) "	1 in 10.
Potassic ferricyanide.	$K_6Fe_2Cy_{12}$.	18.....	" " "	1 in 10.
Ferric chloride.	Fe_2Cl_6.	19.....	" " "	1 in 10.
Acetic acid.	$HC_2H_3O_2$.	20.....	" " "	C. P. 30%.
Calcic sulphate.	$CaSO_4$.	21.....	" " "	Saturated solution.
Mercuric chloride.	$HgCl_2$.	22.....	" " "	" "
Stannous chloride.	$SnCl_2$.	23.....	" " "	Metallic tin boiled with HCl conc. till no more H_2 is evolved. Dilute with 4 parts water. Keep metallic tin in the bottles.
Sodic acetate.	$NaC_2H_3O_2$.	24.....	" " "	1 in 10.
Ammonic sulphate.	$(NH_4)_2SO_4$.	25.....	" " "	1 in 10.
Potassic dichromate.	$K_2Cr_2O_7$.	26.....	" " "	1 in 10.
Magnesic sulphate.	$MgSO_4$.	27.....	" " "	1 in 10.
Lime water.*	$Ca(OH)_2$.	28.....	" " "	Saturated solution.
Calcic chloride.	$CaCl_2$.	29.....	" " "	1 in 10.
Plumbic acetate.	$Pb(C_2H_3O_2)_2$.	30.....	" " "	1 in 10.
Indigo solution.		31.....	" " "	1 part acid indigo extract in 10 c.c.
Ammonic molybdate.		32.....	" " "	Dissolve 1 part molybdic acid in 4 parts NH_3OH. Pour slowly, with constant agitation, into 15 parts HNO_3, sp. gr. 1,200.
Ammonic sulpho-cyanide.	NH_4CNS.	33.....	" " "	1 in 10.
Baric carbonate.	$BaCO_3$.	34.....	" " "	1 in 10, a mixture.
Sodic carbonate.	Na_2CO_3.	37.....	125 c.c. (4 oz.) W. M.	C. P., dry.
Borax.	$Na_2B_4O_7$.	38.....	" " "	Not used.
Phosphorous salt.	$NaNH_4HPO_4$.	39.....	" " "	Not used.
Sodic nitrate.	$NaNO_3$.	40.....	" " "	Pure, crystals.
Potassic cyanide.	KCN (KCy).	41.....	" " "	Pure, sticks.
Ferrous sulphate.*	$FeSO_4$.	42.....	" " "	Pure, crystals.
Test papers.		43.....	" " "	Red and blue litmus paper in strips.
Cobaltic nitrate.	$CO(NO_3)_2$.	Not numbered	125 c.c. (4 oz.) N. M.	1 in 10.

* Stopper greased with mixture of equal parts vaseline and paraffin.

APPENDIX E.

LIST OF APPARATUS

Issued to students of the College of Physicians and Surgeons, New York, for the course in

MEDICAL AND PHYSIOLOGICAL CHEMISTRY.

These are the prices at which students can purchase this apparatus from reliable apparatus dealers like Eimer & Amend, Eighteenth St. and Third Ave., or Emil Greiner, 146 William St., New York.

GLASSWARE.

4	Test-tubes (large)	$0 20
12	" " 5x¾ in.	30
12	" " 4x½ in.	25
1	Wash-bottle, litre, heavy rubber stopper, complete	95
2	Conical glasses, 150 c.c. @ 25c.	50
1	Burette, Binks, 25 c.c., graduated to $\frac{1}{10}$ c.c	1 10
1	Urinometer in case	60
1	Cylinder with foot, 7x1 in.	30
4	Watch-glasses, 2-in.	10
8	Microscope slides, No. 3 (plain glass)	10
1	Pomade jar with cover, 2-inch diameter	10
6	Cover-glasses, No. 2, ½-inch square	10
1	Flask, 50 c.c., measured	25
2	Flasks, Bohemian, 200 c.c. @ 17c.	34
1	Pipette, 5 c.c.	12
1	Thermometer, chemical, 110° C., paper scale	1 00
2	Nests (4 each) Bohemian beakers, plain, Nos. 1, 2, 3, 4 incl. @ 60c.	1 20
3	Funnels (1 each), 2-inch (10c.), 3-inch (15c.), 4-inch (20c.)	45
1	Piece blue glass, 2x3	10
1	" plain glass	05
2	Glass rods	10
1	Glass rod, with platinum wire	50
1	Funnel tube for boneblack	10
1	Dialyzer	20
1	Glass-stoppered, narrow-mouth bottle, 225 c.c. (8 oz.)	20
2	Glass-stoppered, wide-mouth bottles, 25 c.c., containing phenyl hydrazin and sodic acetate @ 25c.	50

PORCELAIN.

1	Mortar and pestle, 4½x4½-in	$0 65
1	Nest of 4 Berlin evaporating-dishes, Nos. 1, 2, 3, 4	1 50

IRONWARE.

1	Ring-stand with 3 rings	70
1	Tripod, Bunsen, heavy	35
2	Iron triangles @ 5c.	10
1	Rat-tail file, 5 in	15
1	Black blowpipe, jap., with tip	30
2	Pieces gauze, iron, 5 inch square	15
1	Copper-water-bath, 5x5½-inch, with rings	1 15
1	Steel forceps, plain, 4 in	10
1	Knife, wood handle, 1 blade	25
1	Tin pan, 6 in	10
2	Bunsen burners @ 40c.	80
18	inches heavy rubber tubing, ¼-in	22

GRANITE IRONWARE.

1	Saucepan with handle	40
1	Cup	30
1	Pan, 7-inch diameter, flat	25

MISCELLANEOUS.

1	Package each white filter paper, { 7-in. (15c.), 9-in. (20c.). 11-in. (22c.), 13-in. (25c.).	82
1	Test-tube holder, wood	15
1	" " rack, 12-13 tubes	40
1	Filter stand, 4 funnels, wood	90
1	Horn spatula, 5 inch, double end	10
2	Test-tube brushes, sponge end @ 10c.	20
2	Towels	20
2	Boxes matches	05
	Total	$20 00

APPENDIX F.

LIST OF EXTRA APPARATUS, CHEMICALS, ETC.,

Required for the different lessons of the course.

LESSON I.

STARCH AND CELLULOSE.

Potatoes. Should be washed first. One for each student.

Corn starch. One tablespoonful for each student.

Potato sections. To be cut with microtome, and kept in water or very dilute alcohol. One for each microscope. Give out during the lesson.

Cupric-hydrate solution. Make by soaking copper strips in strong NH_4OH for a long time; also by ppting $Cu(OH)_2$ from $CuSO_4$ by KOH, filtering, washing (very tedious), and then making saturated solution in strong NH_4OH. Test before using, on filter paper. One 125-c.c. bottle for two desks.

Microscopes. One for every two desks. Extra ones in windows.

Microscope with polariscope attachment. Place on desk or in prominent place with good light. Set up under it a shred of filter paper with some granules of potato starch, both well cut and covered. Also have on hand, if possible,

Barley, rye, wheat, and other *starches* or *flours;* also any *mounted slides* of *starches, grain sections,* etc.

LESSON II.

DEXTRIN AND GLUCOSE.

Dextrin. Two kinds—in grains and in flour. They should give different colors with iodine. If necessary, prepare one as in Lesson III., and stop when the right color is obtained with iodine. One small teaspoonful of each for each student.

Glucose. Common crystallized dextrose. One or two small lumps for each student.

Raisins. One or two for each student.

Grapes. One or two for each student.

Pumice-stone. Two or three small lumps for each student.

Nylander's solution. One bottle (125 c.c.) for two desks. In 100 c.c. water dissolve 10 gms. sodic hydrate (pure in sticks), 2 gms. Rochelle salt (crystals), and 4 gms. bismuth subnitrate. Use rubber stopper, or grease well the glass stopper.

Picric-acid solution. One bottle (125 c.c.) for two desks. Saturated solution of the crystals in water.

Standard glucose solutions. To be given out during the lesson, 40 to 50 c.c. for each student.

Need two solutions, about 1% and 5%. The commercial dextrose is not over 80–85% pure, as a rule.

Also have on hand, if possible,

Molasses, brown sugar, honey, etc., to be tested for glucose.

LESSON III.

CONVERSION OF STARCH INTO DEXTRIN, GLUCOSE, AND MALTOSE.

Corn starch. Big spoonful for each student.

Sand. Wide-mouthed bottle (200 c.c. or more) for each student.

Malt. Teaspoonful for each student.

Nylander's solution. As in Lesson II.

Picric-acid solution. As in Lesson II.

N. B. Fill up the barium-carbonate bottles after this lesson.

LESSON IV.

CANE SUGAR, MILK SUGAR, AND FERMENTATION.

Cane sugar. One small teaspoonful for each student.

Glucose. One small lump for each student.

Milk sugar. Half a teaspoonful for each student.

Cornmeal, rye flour, and malt. These should be given out in the proportions by weight, of 80, 10, and 10 respectively. The proportion of water should be about equivalent to 45 gallons per bushel (56 lbs.) of mixed grain.

In practice we use the following proportions:

Water, 200 c.c. (about half the saucepanful).

Cornmeal, 28.6 c.c.

Rye flour and malt (unground), each 3.3 c.c.

The latter are measured out in marked test-tubes.

Nylander's solution. As in Lesson II.

Picric-acid solution. As in Lesson II.

Yeast. Either half a teaspoonful of fresh ale yeast from a brewery, or ½ or ¼ a cake of compressed yeast, for each student.

Glass tubing. Soft, ¼ inch in diameter; 15 to 18 inches for each student.

Perforated corks. To fit the bottles; small hole through each; should be well soaked. Need one for each student and several over.

Fermentation bottle. 500 c.c. (16 oz.) green-glass, narrow-mouth bottles; one for each student. They need careful cleaning after the next lesson.

N. B. After this lesson, refill the lime-water bottles.

LESSON V.

ALCOHOL, CARBONIC ACID, AND YEAST.

Molybdic-acid solution. One bottle for two desks. Keep in earthenware saucers. Solution consists of 1 part molybdic acid in 10 parts by weight of concentrated sulphuric acid.

Pumice-stone. Two or three small pieces for each student.

Perforated corks. Same as in last lesson.

Glass tubing. Same kind as in last lesson; need about 30 inches for each student. Have several over.

The students should keep for future lessons a good distilling-tube and a good fermentation tube in each desk.

Microscopes. One in each window, and some on spare desks in the laboratory.

N. B. This is the first lesson where the student uses the

Platinum wire on glass rod. It is often well to give these out specially at this lession, and to emphasize their value.

N. B. Each student must set out a small evaporating-dish for next lesson.

LESSON VI.

FATS AND SOAPS.

Beef fat. Kidney fat is best; about 1 lb. for 20 men; give small lump to each student.

Pork fat. Same as above.

Soft soap. About 50 c.c. for each student.

Prepare beforehand in large agateware dish, by boiling lard with potash and water (proportions: 2 lbs. lard, 6 oz. common potassic hydrate, 1 gallon water) for three or four hours, and diluting with two gallons of water.

Castor oil. Common; give 10 c.c. to each student. Put it in a small evaporating-dish.

Sodic-hydrate solution. Saturated water solution containing 30 to 32% common sodic hydrate. Give out during the lesson, 3 c.c. for the above castor oil.

Copper wire. Fine; two pieces, 6 to 8 inches long, to each student; have some over.

Muslin. Common; cheese cloth is sometimes preferable; give piece 6 to 8 inches square to each student.

Melting-point tube. One to each student. Make them by drawing out to a closed point pieces of ¼-inch glass tubing, 1½ or 2 inches long.

LESSON VII.

BUTTER AND OILS.

Butter. Give to each student one lump of about 5 gms. and a little lump alongside for the other test.

Oleomargarin oil. Obtained at a large slaughter-house, like Eastman & Co., foot of West Fifty-ninth Street, New York. Give a quarter teaspoonful to each student.

Alcoholic-potash solution. ½ normal: 28 gms. stick potassic hydrate in 1 litre alcohol. Give each student about 40 c.c. (in the 50 c.c. flask).

Perforated corks. As in Lessons IV. and V.

Straight glass tubing. As before. About 18 inches for each student.

Sand in bottles. As in Lesson III.

Pumice-stone. As in Lessons IV. and V.

Gasolene. Small bottle for every two desks.

Glycerin. " " "

Olive oil. " " "

Castor oil. " " "

Cod-liver oil. " " "

LESSON VIII.

EGG AND SERUM ALBUMIN AND VITELLIN.

Eggs. One for each student, and a few extra.

Egg albumen, dry. A good teaspoonful for each student.

Serum albumin, dry. A good teaspoonful for each student.

Broken glass. Half a teaspoonful for each student.

Empty bottle. 12 to 16 oz., glass or cork stoppered; one for each student.

Muslin or cheese cloth. Piece 4 inches square for each student.
Corks. To fit test-tubes, with good-sized holes, as in former lessons.

Also the following reagents, either on every desk or on every second desk:

Picric acid. Saturated aqueous solution.
Sodium sulphate. Saturated aqueous solution.
Glacial acetic acid.
Ether. Common.
10% *sodium-chloride solution.*
Millon's reagent. This is prepared by dissolving one part, by weight, of metallic mercury in two parts of concentrated nitric acid (sp. gr. 1.42), and diluting the solution with twice its bulk of water.

It works best if the reagent has been prepared for some time.

LESSON IX.

CRYSTALLIN, MYOSIN, ACID AND ALKALI ALBUMIN.

Chopped meat. A good spoonful for each student.
Bullock's eyes. One spoonful for each student.
Egg albumen, dry. A quarter of a teaspoonful for each student.
Muslin or cheese cloth. As in last lesson.
Sodic-hydrate solution. As in Lesson VI.; give out 10 c.c. or so to each student during the lesson.

Also, as in last lesson,

Picric-acid solution.
Millon's reagent.
10% *sodium-chloride solution.*
$\frac{1}{2}$% *sodium-chloride solution.*

N. B. The students should be directed to bring some scalpels or dissecting-scissors at this lesson, or else some should be lent from the laboratory supplies, so that they can dissect out carefully the lenses from the eyes.

LESSON X.

SYNTONIN, GLUTIN, SULPHUR IN PROTEIDS.

$\frac{5}{10}$% *hydrochloric acid.* 1 bottle for two desks. 5 c.c. HCl conc. in 1 litre water.
Nylander's solution. As in Lesson II.
Millon's reagent. As in Lesson VIII.

Washed muscle. Chopped meat soaked for two or three hours and well squeezed; half a teaspoonful for each student.
Muslin. As in Lesson IX.
Wheat flour. 1 tablespoonful for each student.
Egg albumen. Half a teaspoonful for each student.
Nitro-prusside of sodium. A few crystals for each student.

LESSON XI.

OXYGEN, HYDROGEN, CHLORINE, AND HYDROCHLORIC ACID.

Potassium chlorate. Powdered; small teaspoonful for two students.
Charcoal. Small piece, 1½ by ¼ inch or so, for two students.
Copper wire. 6 inches for two students.
Wide-mouthed 8 or 10 oz. bottle. Smooth top, for two students.
Delivery tube. Left over from Lesson IV., for two students.
Evolution flask. Heavy glass, flat bottomed, 8 or 10 oz., with two-hole rubber cork, thistle tube and exit tube, for two students.
Ignition tube. ¼-inch hard glass, 4 to 6 inches long, drawn to point, for two students.
Piece rubber tubing. To connect ignition to exit tube, 2 to 3 inches for two students.
Zinc turnings. Old, from Lesson XVI., one tablespoonful for two students. Throw away after lesson, as containing arsenic.
White arsenic. As_2O_3; give out a pinch during lesson, for two students.
Muscle from dissecting-room. Give small piece, during lesson, for two students.
Bleaching powder. Large teaspoonful for two students.
Congo paper. A few strips for two students.
Manganese dioxide. Half a teaspoonful for two students.
Copper foil. Small bright strip, 1 by ½ inch, for two students.

LESSON XII.

SULPHURIC, CARBONIC, AND NITRIC ACIDS.

Limestone. Two or three lumps for each student.
Saltpetre. Common; Chili, or potash; one teaspoonful for each student.
Brucine. Crystals; give a little during lesson to each student.

Aniline. Give ½ inch in a small test-tube during lesson to each student.

Carbolic acid. Strong; ½ inch in a small test-tube for each student.

Copper tacks. Two for each student.

Iron nail. One for each student.

Extra corks. Put dishes of these in two or three places in the laboratory.

Paraffin. Keep dishes of melted paraffin near the corks.

Fine iron wire. Keep few pieces, 4 inches long, near the corks.

LESSON XIII.

PHOSPHORIC ACID; IRON AND ALUMINIUM.

Iron nails. Two for each student.

Aluminium foil. One strip, 1 by ½ inch or so, for each student.

Alum. Half a teaspoonful for each student.

Aluminic sulphate. Half a teaspoonful for each student.

Potassic sulphate. Half a teaspoonful for each student.

Tannin. A quarter of a teaspoonful for each student.

Blowpipe charcoal. Half a piece for each student.

Cochineal solution. } Give out, during the lesson, in small test-
Litmus solution. } tubes, an inch or so of each for each student.

Microscopes. In each window and on empty desks in the laboratory.

LESSON XIV.

CALCIUM AND MAGNESIUM.

Limestone. One small lump for each student.

Quicklime. Fresh and "quick," one lump for each student.

Magnesium tape or wire. One inch or so for each student.

Blowpipe charcoal. Same as in last lesson.

Dolomitic limestone, stained slightly with ferric chloride. One small lump for each student.

Microscopes. As in the last lesson.

N. B. In the course of this and the following lesson it is interesting to demonstrate to the students some of the different varieties of *spectra*, *e.g.*, the continuous spectrum of a gas jet, the dark-line or solar spectrum, and the bright-line *spectra* of some of the common metals, such as sodium, potassium, barium and calcium. This can be done quite easily, with the aid of a good direct-vision spectroscope.

LESSON XV.

THE ALKALINE METALS, ACIDIMETRY AND ALKALIMETRY.

Metallic sodium.
Metallic potassium.
A small piece of each must be given to each student during the lesson. To avoid accidents, it is best to drop the metal directly into the saucepan, one-third full of water, and cover it over at once with the glass plate.

Lithium carbonate. One-quarter teaspoonful for each student.

Wood ashes. A tablespoonful for each student.

Tumeric paper. A few strips for each student.

Platinic chloride. See that ¼ or ½ an inch of this solution is in each reagent bottle.

Microscopes. As in Lesson XIII. Give out during the lesson.

Standard alkali solution. A normal aqueous solution of potassic hydrate containing, in 1 litre, 56 gms. of pure KOH.

Standard acid solution. Sulphuric or hydrochloric acid diluted with water till 1 c.c. of it is exactly equivalent to 1 c.c. of the standard alkali solution. Allow from 50 to 75 c.c. of each for each student.

Also give out to each student, in test-tubes, half an inch or so each of

Potassium antimonate. Saturated aqueous solution.

Phenol phthaleïn. Strong solution in dilute alcohol.

Orange No. 2. Strong aqueous solution.

N. B. Three (3) beakers should be left out on each desk in preparation for Lesson XVI.

LESSON XVI.

WATER ANALYSIS.

Well-water. Either from a contaminated well, *e.g.*, an artesian well in New York City, or made by "doctoring" ordinary water. It should give fair tests for lime, sulphates, free ammonia and nitrites, and should contain to the gallon over five (5) grains of sodium chloride, and five (5) grains of total hardness, calculated as calcium carbonate.

Allow 150 to 200 c.c. for each student.

Standard silver-nitrate solution. Aqueous solution containing 2½ gms. pure $AgNO_3$ to the litre.

Allow 30 or 40 c.c. for each student.

Standard soap solution. For the preparation see page 148.

Allow 30 or 40 c.c. for each student.

Mineral water. This can be made up in the laboratory, or can use any convenient mineral water. Syphons of Vichy with lithia from a reliable dealer* in artificial mineral waters are extremely convenient and not expensive. Allow 50 or 60 c.c. for each student.

For every two desks set out a small bottle of each of the following three solutions:

Nessler's solution. Dissolve 50 gms. potassic iodide in hot water; add strong mercuric-chloride ($HgCl_2$) solution till the red ppt. formed just redissolves; add 200 gms. KOH, dilute to 1 litre, add 5 c.c. $HgCl_2$, and let settle.

Naphthylamin hydrochlorate. 10 gms. β naphthylamin, 10 c.c. HCl conc.; water to 250 c.c.

Sulphanilic acid, or, if more readily obtained, *sulphanilate of soda.* A saturated aqueous solution.

Give out during the lesson, to each student,

Phenol. (Carbolic acid.) Half an inch in a small test-tube.

Potassic chromate. Half a test-tube full of a saturated aqueous solution.

Also set out

Wide-mouthed bottle, 8 to 12 oz.; filled a quarter full of zinc turnings. One for each student.

LESSON XVII.

BONE.

Dry bone. Several small pieces, enough to fill one large test-tube, for each student.

Also have a lot over.

Macerated bone. Bones to be soaked in 25% HCl for two or three days. Chicken and turkey bones best. One or two for each student.

Funnel tube. A small test-tube drawn to a point leaving hole $\frac{1}{10}$ inch or so in diameter, may be issued with apparatus. One for each student.

* Hygeia Sparkling Distilled Water Co., 347 West Twelfth Street; or Carl H. Schultz, East Twenty-fourth Street, New York City.

Boneblack. Coarse and well washed. Enough to fill the funnel tube.

Blowpipe charcoal. Same as in Lesson XIV.

Glue. Two or three small pieces for each student.

Tannic acid. Half a teaspoonful for each student.

N. B. See that the *barium carbonate* bottles are refilled.

N. B. One beaker and one small evaporating-dish should be left out on each desk for Lesson XVIII.

LESSON XVIII.

MILK AND KOUMYSS.

Lactometers and cylinders. Set these in sets of five each, labelled carefully, at convenient places in the laboratory. Arrange as follows: 1°, skim milk; 2°, whole milk; 3°, watered milk (about 25% of water); 4°, cream (this should be a few points lighter than 3°, and yet the lactometer must not touch the bottom); 5°, water, in a large and deep cylinder. If few lactometers are available, leave out instrument in 5°, letting the cylinder stay.

N. B. Must have the skim milk *heavier* than the whole milk.

Whole milk. Fair quality cow's milk, enough for the cylinders.

Skim milk. Generally have to set enough for the lesson, the day before, in a large jar, and syphon off from the cream.

Need enough for the cylinders and to allow each student 75 c.c. or so.

Cream. Need enough for cylinders and to allow each student 15 to 25 c.c.

Rennet. Need it very strong. Best make up fresh from *rennet tablets.*

Alcoholic potash. Same as for Lesson VII. Allow 15 or 20 c.c. for each student.

Millon's reagent. Same as in Lesson VIII. and others.

Koumyss. May make in laboratory or may buy it in bottles. Need 30 or 40 c.c. for each student. Give it out during lesson, and make the students notice the carbon dioxide evolved.

Corks. Same as in Lesson V.

Pumice-stone. Same as in Lesson V.

Molybdic-acid solution. Same as in Lesson V.

LESSON XIX.

BLOOD.

Defibrinated blood. Prepared by whipping or squeezing the fibrin out of fresh blood; 25 or 30 c.c. for each student.

Blood serum. Prepared by letting blood coagulate in a pan or dish, carefully loosening the edges of the clot, and letting it stand for 24 hours; 30 to 50 c.c. for each student.

Fibrin. Should be thoroughly washed; can be prepared fresh each time, or can be kept indefinitely either dry or in alcohol. Half a teaspoonful for each student.

Limestone. Three or four small lumps for each student.

10% sodium-chloride solution.
½% sodium-chloride solution.
Gasolene.
} As in Lessons VII., VIII., and IX.

Tartaric acid. Dry. Half a teaspoonful for each student.

Microscopes. In the windows and on empty desks.

LESSON XX.

BLOOD (*Continued*) AND BILE.

Dried blood. Half a teaspoonful for each student.

Cloth stained with blood. (a) old, (b) fresh; a small piece or two of each for each student.

Potato. One-half for each student.

Gall stone. Either a small piece or a pinch of powdered stone for each student.

If it is impossible to obtain this, a little cholesterin can be given.

Bile. Fresh ox's bile. 30 to 40 c.c. for each student.

Glacial acetic acid. Glass-stoppered bottle for two desks.

½% sodium-chloride solution. As in last lesson.

Egg albumen. A quarter-teaspoonful for each student.

Glycogen. This should be prepared by rapidly taking the livers from well-fed rabbits, plunging them in boiling water, hashing them fine, extracting them with boiling water, and running the extract into an excess of strong alcohol. A pinch of the precipitate is enough for two students.

Microscopes. In the windows and on empty desks.

Also give out a few drops, in test-tubes, of

Tincture of guaiacum. This ought to be fresh.

Hydrogen peroxide solution. This must be fresh for each lesson.

25% sugar solution.

N. B. Set out four beakers for the next lesson.

LESSON XXI.

DIGESTION.

Peptone solution. May try some of the artificial peptones in the market; but most of the tests will succeed with strong solutions of Liebig's, Armour's or Valentine's extract of beef.

Need 30 to 40 c.c. of the solution for each student.

Artificial gastric juice. To 1 litre water add 5 c.c. C.P. hydrochloric acid, ½ gm. lactic acid, ½ gm. hydro-disodic phosphate, and from 10 to 25 gms. of pure pepsin (Fairchild or Parke, Davis & Co.). Test carefully before using.

Need from 50 to 75 c.c. for each student. Can make up others, for practice, with different proportions.

Standard alkali. $\frac{1}{10}$ normal. Made by diluting standard alkali from Lesson XV. ten times with water.

Need 40 to 50 c.c. for each student.

Pancreatic juice. To 1 litre water add 5 gms. dry sodic carbonate, 5 gms. sodic chlorine, and from 10 to 25 gms. of dry pancreatin (Parke, Davis & Co. or Fairchild).

Test before using. Need 25 to 30 c.c. for each student.

Milk. Need 25 to 30 c.c. for each student.

Picric-acid solution. As in Lesson II.

Ammonic sulphate. Crystals. Half a teaspoonful for each student.

Tannin. Dry. A quarter-teaspoonful for each student.

Fibrin. As in Lesson XIX. Half a teaspoonful for each student.

Calcium carbonate. Powdered. Half a teaspoonful for each student.

Starch. Half a tablespoonful for each student.

Sand. In bottle as in Lesson III.

Wire gauze. Extra piece, to fit in bottom of cup, for each student.

Parchment paper. One piece, 5 to 6 inches square, for each student.

Dialyser ring. About 3 inches in diameter, 1½ or 2 inches in width, with raised rims. On the apparatus list.

String. One piece, 10 to 12 inches long, for each student.

Microscopes. In each window and on empty aisles.

Also give out in small test-tubes, during the lesson, a quarter or half inch of each of the following solutions:

Phenol-phthaleïn solution. As in Lesson XV.

Resorcin and sugar solution. 5 gms. resorcin and 3 gms. sugar in 100 gms. diluted alcohol.

Phloroglucin and vanillin solution. See page 222. This is extremely expensive and may be left out.

Leucin and tyrosin solution. Made by digesting for 3 or 4 days some fibrin in pancreatic juice. Test before using.

LESSON XXII.

GENERAL PROPERTIES OF URINE.

Need samples of normal and pathological urines, properly marked, in sufficient abundance to more than go round the class. It is most convenient, usually, to leave the bottles for these, duly corked and labelled, at the hospitals over night, and to call for them between 12 and 1 P.M. the next day.

It is important to explain thoroughly to the students the objects and importance of this lesson, before they begin work, and to arrange that the samples of urine pass from student to student.

If pressed for time, it is possible to incorporate Lesson XXIV. with this.

LESSON XXIII.

UREA AND URIC ACID.

Need several specimens of normal and pathological urines; also:

Hypobromite solution. Dissolve 100 gms. stick sodic hydrate in 250 c.c. water, and add 25 c.c. of bromine.

Must make it up fresh. Need 100 to 150 c.c. for each student. Give out in corked bottles.

Concentrated urine. Evaporate strong or normal urine to $\frac{1}{4}$ of its bulk. Give 15 to 20 c.c. to each student.

Snake's excrement. Either powdered or in small lumps. A pinch for each student.

Can, if necessary, substitute a mixture of uric acid and ammonium urate.

Oxalic acid. Crystals. A quarter-teaspoonful for each student.

Mercuric nitrate. Give out a little in a test-tube to each student.

Doremus' urea apparatus. } Should have one of each for every
Marshall's urea apparatus. } two students.

Microscopes. In each window, and wherever possible along the aisles. Should be put out after the hypobromite fumes have disappeared.

LESSON XXIV.

ALBUMIN IN URINE.

Need several specimens of urine illustrating the different varieties of nephritis, and also, if possible, of temporary albuminuria. Also, specimens of urine may be doctored with small amounts of serum albumin.

LESSON XXV.

GLUCOSE IN URINE.

Wherever possible provide specimens of diaoetic urines. If this is impossible, must prepare specimens by diluting with water and by adding from 5 to 10% of glucose. It is hard in this way to make a good imitation of true diabetic urine, because the specific gravity cannot be raised sufficiently by glucose alone.

Fermentation bottle. As in Lesson IV.

Yeast. As in Lesson IV.

LESSON XXVI. TO XXX.

MICROSCOPICAL EXAMINATIONS OF URINE.

Most of the crystalline sediments can be readily obtained by keeping specimens from the last four lessons and allowing them to ferment.

The casts and similar organized deposits, and also the rarer forms and varieties of crystals, are often well worth preserving from one term to another. This can be done temporarily by adding to the urine chloral, carbolic acid, salicylic acid, or other antiseptics; or permanently by the replacing of the urine by some preserving fluid, such as a solution of glycerin in water, sp. gr. 1020, to which 1% of carbolic acid is added, or a solution of potassium acetate, sp. gr. 1016, containing ½% of carbolic acid.

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